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Pathogenesis of Virus Infections

Pathogenesis refers to the sequence of events during the course of an infection within the host, and the mechanisms giving rise to these events. It includes entry of the virus into the body, multiplication and spread, the development of tissue damage, and the production of an immune response. It encompasses the appearance of clinical signs and symptoms, the eventual resolution of the infection and, in most cases, virus elimination. Understanding viral disease pathogenesis requires knowledge of each of the stages of infection and an awareness of the mechanisms involved. The pattern of pathogenetic events accompanying infection is remarkably consistent and specific for each individual virus. There may be variation from individual to individual in the severity and/or the duration of these events, but a sound knowledge of the typical sequence associated with each infection is crucial in both making an accurate diagnosis and recommending the appropriate treatment.

In this chapter the mechanisms underpinning the initiation, establishment, and outcome of typical virus infections are described.

For the successful initiation of an infection, three requirements must be satisfied:

1. There must be an inoculum containing sufficient viable virus to establish an infection;
2. Virus must first reach and interact with susceptible cells capable of supporting virus replication;
3. The host innate immunity and pre-existing adaptive immunity must be insufficient to immediately abort the infection.

THE INCOMING VIRUS INOCULUM

The *infective titer* of a sample of virus can be measured by titration in a defined experimental assay system (e.g., in cell culture or an animal model), by determining the highest dilution (i.e., the lowest concentration) of the sample that can still initiate an infection, for example, that will infect 50% of the cell cultures (tissue culture infective dose 50, TCID₅₀) or animals (lethal dose 50, LD₅₀; or infective dose 50, ID₅₀) used for measurement. It is expressed as a

number per volume, for example, 100 LD₅₀ per ml. One LD₅₀ generally represents many more than a single virus particle as (1) usually many of the incoming virus particles are non-infective due to defective assembly, genetic errors, or inactivation caused by environmental conditions, etc., and (2) usually many or most interactions between infective particles and cells do not lead to a productive infection at the tissue/organ/individual level.

Further, the “*infective titer*” of a virus sample is not an absolute value, but varies according to the assay system used—there are examples where humans may be infected by samples assayed by a particular laboratory method as containing less than one infectious unit—the human being more sensitive than the laboratory assay. Clearly the volume of the inoculum is important. In blood transfusion, a 500 ml unit of blood may contain an infecting dose even if the virus concentration is low (and transfusion recipients are often also debilitated by a major illness or injury); hence, highly sensitive PCR-based testing of every unit of donated blood is often necessary, as for example, when West Nile virus was first introduced into the United States.

Importantly, inactivation of a virus-containing sample by environmental conditions or by a disinfectant is not an all-or-none process, but a progressive stepwise reduction in virus titer over time as more and more virions become inactivated.

ROUTES OF ENTRY—A MAJOR FACTOR GOVERNING THE SPECIFIC PATTERN OF INFECTION

The surfaces of the body are lined by an almost continuous layer of epithelium—the epidermis or skin externally, and the various kinds of mucosae internally. Any incoming virus needs to be taken up by, and/or pass through, the cells making up this physical barrier. Different viruses have evolved distinctive mechanisms and routes for crossing this first barrier. The route preferred by a particular virus is a significant determinant of the type of damage and disease seen, and also is a major factor determining epidemiological characteristics (Fig. 7.1).

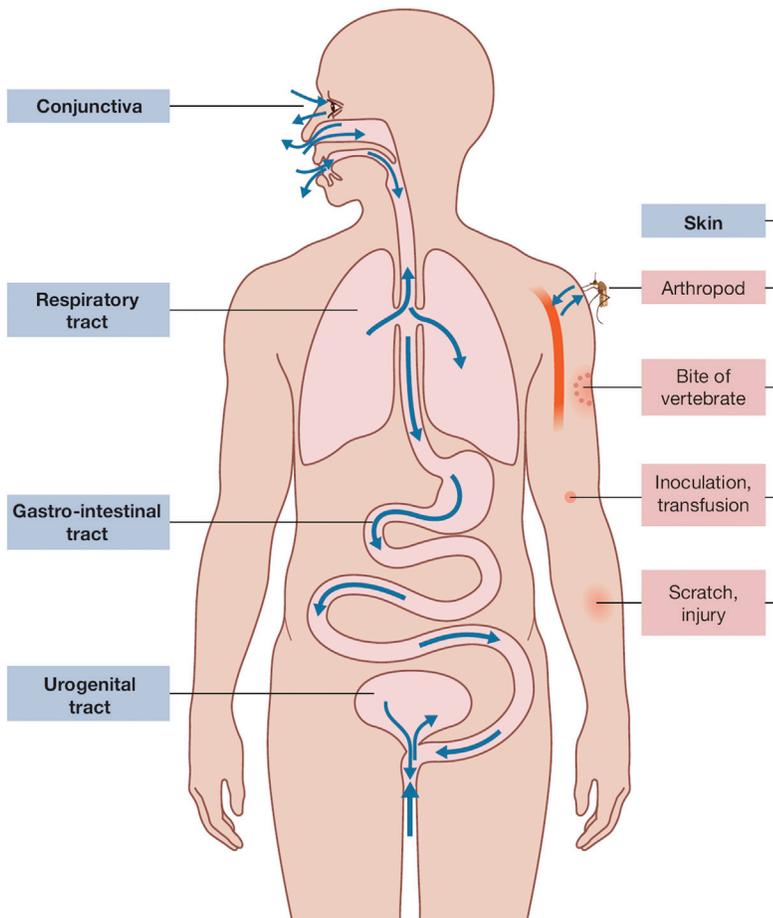


FIGURE 7.1 Routes of virus entry—the various surfaces of the body, one of which must be breached in order for infection to occur. *Reproduced from Flint, S.J., et al., 2009. Principles of Virology, third ed., Vol. II, p. 348. ASM Press, Washington, DC, with permission.*

Following entry by either of these routes, virus replication may remain *localized* to the site of entry or may spread more widely through body compartments becoming *systemic*. Clinical signs and symptoms are often, but not always, related to the site(s) of virus replication.

Virus Entry via the Respiratory Tract

The cells lining the respiratory tract can support the replication of many viruses. The surface of the respiratory tract is protected by two cleansing systems: (1) a blanket of mucus produced by goblet cells, kept in continuous flow by (2) the coordinated beating of cilia on the epithelial cells lining the upper and much of the lower respiratory tract. Inhaled virus particles deposited on this surface are trapped in mucus, carried by ciliary action (the “*mucociliary escalator*”) from the airways and nasal cavity to the pharynx, and then swallowed or coughed out. Inhaled droplets of 10 μm or more in diameter are usually deposited on the nasal mucosae covering the nasal turbinates: these project into the nasal cavity and act as baffle plates. Droplets of 5 to 10 μm in diameter are often carried to the trachea and bronchioles, where they are usually trapped in the mucus

blanket. Droplets of 5 μm or less may be inhaled directly into the lungs, and some may reach the alveoli, where contained virus particles may infect alveolar epithelial cells directly, causing viral pneumonia (interstitial pneumonia) (Fig. 7.2, Fig. 39.1).

The respiratory tract is, overall, the most important entry site of viruses into the body (Table 7.1). All viruses that infect the host via the respiratory tract do so by attaching to specific receptors on epithelial cells. Following initial respiratory tract infection, many viruses remain localized (e.g., rhinoviruses, parainfluenza, and influenza viruses), whereas others become systemic (e.g., measles, varicella-zoster, and rubella viruses).

Virus Entry via the Alimentary Tract

Many viruses are acquired by ingestion. Virus particles may either be swallowed and reach the stomach and intestine directly, or first infect cells in the oropharynx with progeny eventually carried into the intestinal tract. The esophagus is rarely infected, probably because of its tough stratified squamous epithelium and the rapid passage of swallowed material over its surface. The intestinal tract is protected by mucus, which may contain specific secretory antibodies

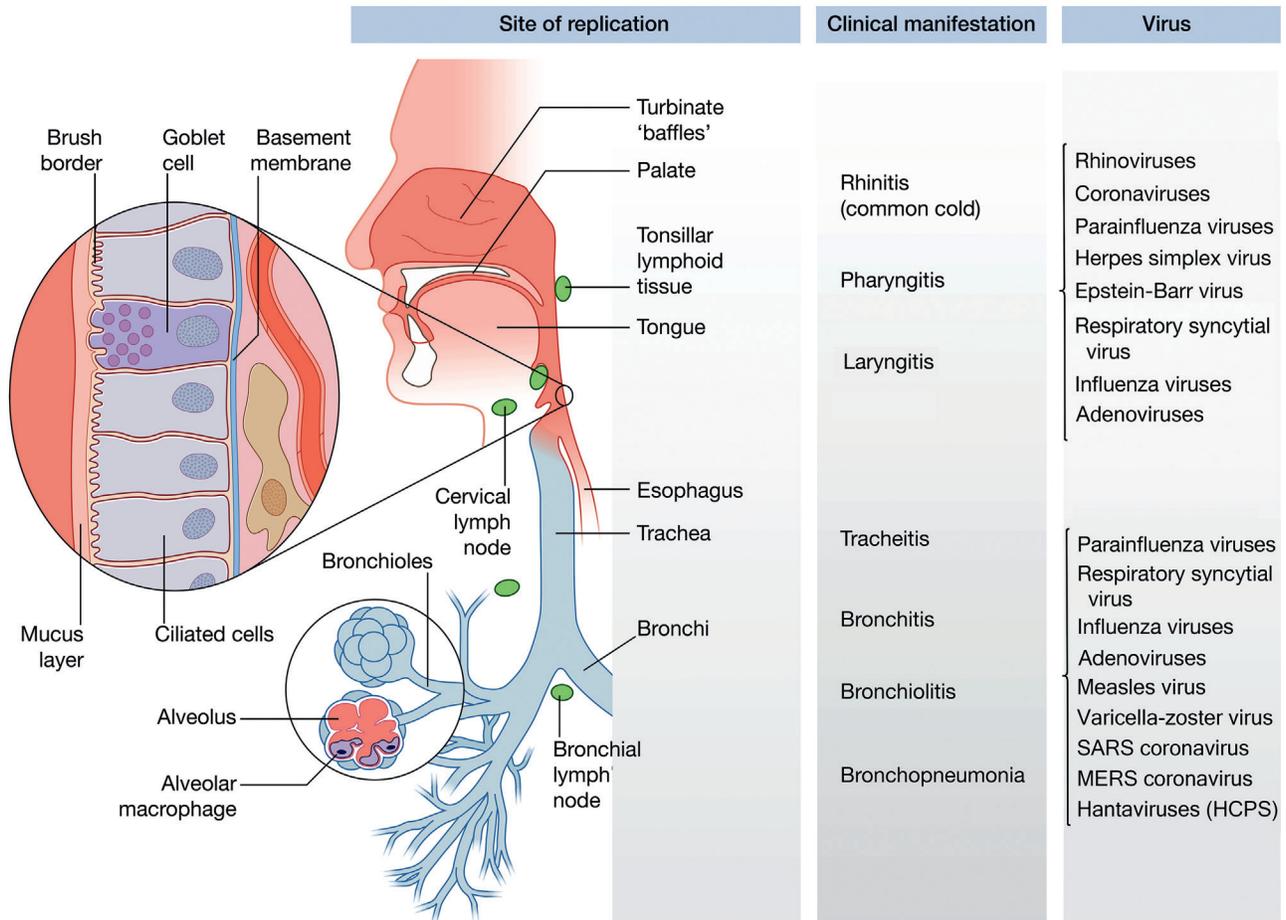


FIGURE 7.2 Sites of viral entry in the respiratory tract. (Left) A detailed view of the ciliated pseudostratified columnar respiratory epithelium. A layer of mucus, produced by goblet cells, is a formidable barrier to virion attachment. Virions that pass through this layer may multiply in the ciliated cells or pass between them, reaching another physical barrier, the basement membrane. Beyond this are tissue fluids from which particles may be taken into lymphatic capillaries and reach the blood. Local macrophages patrol the tissue fluids in search of foreign particles. (Right) Different viruses replicate and cause clinical disease most prominently at different levels of the respiratory tract. *Originally from Mims, C.A., 1977–2008. Pathogenesis of Infectious Disease (through six editions). London, UK: Elsevier/Academic Press. Adapted by Nathanson, N. (ed.); and then adapted from Flint, S.J., et al., 2009. Principles of Virology: Pathogenesis and Control, third ed., ASM Press, Washington, DC.*

(IgA), but the directional peristaltic movement of gut contents provides many opportunities for virus particles to contact susceptible epithelial cells. Viruses may also be taken up by the M cells, specialized transporter cells that overlie Peyer’s patches in the ileum, from where they may be passed to adjacent mononuclear cells and be carried away to draining lymph nodes and then enter the bloodstream (Table 7.2).

Many viruses entering the intestinal tract are inactivated by either acid in the stomach or the detergent activity of bile and proteolytic enzymes in the small intestine. In general, viruses causing intestinal infection, such as rotaviruses, caliciviruses, and enteroviruses are acid- and bile-resistant. However, there are acid- and bile-labile viruses that cause important intestinal infections; for example, coronaviruses. Additionally, viruses are protected during passage through

the stomach of babies by the buffering action of milk. Some enteric viruses not only resist inactivation by proteolytic enzymes in the stomach and intestine, but infectivity may actually be increased by such exposure. The cleavage of a viral outer capsid protein by intestinal proteases enhances the infectivity of rotaviruses and some coronaviruses.

Rotaviruses, caliciviruses (noroviruses), and astroviruses are major causes of viral gastroenteritis and diarrhea. In contrast some of the enteroviruses (e.g., polioviruses) and hepatitis A and E viruses gain entry via ingestion and are important causes of infection but do not produce clinical signs referable to the intestinal tract. The emergence of AIDS has drawn attention to the importance of the rectum as a route of viral entry—HIV as well as other sexually transmitted agents may gain entry through damaged rectal mucosae.

TABLE 7.1 Viruses that Initiate Infection of Humans via the Respiratory Tract

With the Production of Local Respiratory Symptoms	
<i>Picornaviridae</i>	Rhinoviruses, some enteroviruses
<i>Coronaviridae</i>	SARS CoV, MERS CoV, human coronaviruses OC43, 229E, NL63
<i>Paramyxoviridae</i>	Parainfluenza viruses, respiratory syncytial virus, human metapneumovirus
<i>Orthomyxoviridae</i>	Influenza virus
<i>Adenoviridae</i>	Most types
Producing Generalized Disease, Usually without Initial Respiratory Symptoms	
<i>Paramyxoviridae</i>	Mumps, measles viruses
<i>Togaviridae</i>	Rubella virus
<i>Herpesviridae</i>	Varicella virus
<i>Picornaviridae</i>	Some enteroviruses
<i>Polyomaviridae</i>	Polyomaviruses
<i>Parvoviridae</i>	B19 parvovirus
<i>Bunyaviridae</i>	Hantaan virus
<i>Arenaviridae</i>	South American hemorrhagic fever viruses
<i>Poxviridae</i>	Variola virus (smallpox is now extinct)

Virus Entry via the Skin

The outer keratinized layer of the skin (stratum corneum) is normally impenetrable to viruses unless it is breached mechanically, either by direct trauma, by insect or animal bites, or various inoculation or transfusion procedures (Table 7.3, Fig. 7.3).

Virus replication may then remain localized either in cells of the epidermis (papillomaviruses) or in underlying dermal cells. Alternatively, virus progeny produced within the dermis may be carried by the bloodstream, lymphatics, or nerves to more distant sites. Virus may also be taken up by dendritic cells (Langerhans cells) in the skin and then be transported directly to local lymph nodes. If local virus replication remains confined to the epidermis and does not breach the basement membrane (e.g., herpes simplex virus), scarring of the skin surface is unlikely, whereas if the basement membrane is significantly damaged (e.g., human monkeypox), scarring is the likely result (Table 7.3).

TABLE 7.2 Viruses that Initiate Infection of Humans via the Alimentary Tract

Via Mouth or Oropharynx	
<i>Herpesviridae</i>	Herpes simplex virus, EB virus, cytomegalovirus, HHV6
Via Intestinal Tract	
Producing enteritis	
<i>Reoviridae</i>	Rotaviruses
<i>Caliciviridae</i>	Noroviruses, sapoviruses
<i>Astroviridae</i>	Human astroviruses (HAstV)
<i>Adenoviridae</i>	Some adenoviruses especially HAdV-40, HAdV-41
Producing generalized disease, usually without alimentary symptoms	
<i>Picornaviridae</i>	Many enteroviruses including polioviruses, hepatitis A virus
<i>Hepeviridae</i>	Hepatitis E virus
Usually symptomless	
<i>Adenoviridae</i>	Some adenoviruses
<i>Picornaviridae</i>	Some enteroviruses
<i>Reoviridae</i>	Reoviruses

Virus Entry via the Genitourinary Tract

The genitourinary tract is protected by a mucosal lining, by mucus, and by the low pH of the vagina. However, minute tears or abrasions to the vaginal, rectal, and urethral epithelium during sexual activity can facilitate virus entry (e.g., papillomaviruses). Entry by this route is also facilitated by the exchange of bodily fluids that occurs during sexual activity.

Herpes simplex virus 2 and papillomaviruses produce local lesions on the genitalia and perineum from where they may be transmitted by contact. In contrast many other viruses, for example, HIV-1 and 2, human T-lymphotropic viruses 1 and 2 (HTLV-1 and 2), and hepatitis B and C viruses, do not produce local lesions but are sexually transmitted.

Virus Entry via the Eyes

The conjunctiva, although much less resistant to viral invasion than the skin, is constantly cleansed by the flow of secreted tears and is regularly wiped by the eyelids. Infection is more likely to be introduced if abrasions to the conjunctiva or cornea are present, for example, in dusty environments. Virus can reach the eye by aerosol, by rubbing with

TABLE 7.3 Viruses that Initiate Infection of Humans via the Skin, Genital Tract, or Eye

Route	Family	Virus
Skin		
Minor trauma	<i>Papillomaviridae</i>	Many types of papillomaviruses
	<i>Poxviridae</i>	Molluscum contagiosum, cowpox, orf, milkers' nodes viruses
	<i>Herpesviridae</i>	Herpes simplex viruses
	<i>Hepadnaviridae</i>	Hepatitis B virus
Arthropod bite (mechanical) Arthropod bite/Mechanical	<i>Poxviridae</i>	Tanapoxvirus
	<i>Togaviridae</i>	Many alphaviruses
Arthropod bite (with replication in the arthropod) Arthropod bite/Biological	<i>Flaviviridae</i>	Many flaviviruses
	<i>Bunyaviridae</i>	La Crosse, sandfly fever, Rift Valley fever viruses
	<i>Reoviridae</i>	Colorado tick fever virus
	<i>Rhabdoviridae</i>	Rabies virus
Animal bite	<i>Herpesviridae</i>	Herpes B virus
	<i>Hepadnaviridae</i>	Hepatitis B virus
Injection, inoculation	<i>Flaviviridae</i>	Hepatitis C virus
	<i>Retroviridae</i>	HIV, HTLVs
	<i>Herpesviridae</i>	CMV, EBV
	<i>Filoviridae</i>	Ebola virus
	<i>Papillomaviridae</i>	Genital types of papillomaviruses
Genital tract	<i>Herpesviridae</i>	Herpes simplex viruses
	<i>Retroviridae</i>	HIV, HTLV-1
	<i>Hepadnaviridae</i>	Hepatitis B virus
	<i>Flaviviridae</i>	Hepatitis C virus
	<i>Adenoviridae</i>	Several adenoviruses
Conjunctiva	<i>Picornaviridae</i>	Enterovirus 70
	<i>Herpesviridae</i>	Herpes simplex viruses
	<i>Poxviridae</i>	Vaccinia virus

contaminated fingers, during ophthalmic procedures with improperly sterilized instruments, or from swimming pool water. Patterns of disease produced include conjunctivitis (e.g., some adenoviruses, influenza viruses, South American arenaviruses, and enteroviruses), and recurrent keratitis (inflammation of the cornea, caused by herpes simplex and several other viruses). Macular involvement is a common feature in Rift Valley fever. Rarely, infection can spread systemically following entry via the eyes, for example, paralysis following enterovirus 70 conjunctivitis. There

are reports of Marburg and Ebola viruses persisting in the anterior chamber of the eye long into convalescence.

Vertical Transmission

There are three situations where a virus infection can be transmitted from the mother to her fetus or newborn infant.

1. Transmission of viral genomic DNA encoded in the germ-line or as episomes in ova or sperm may predispose

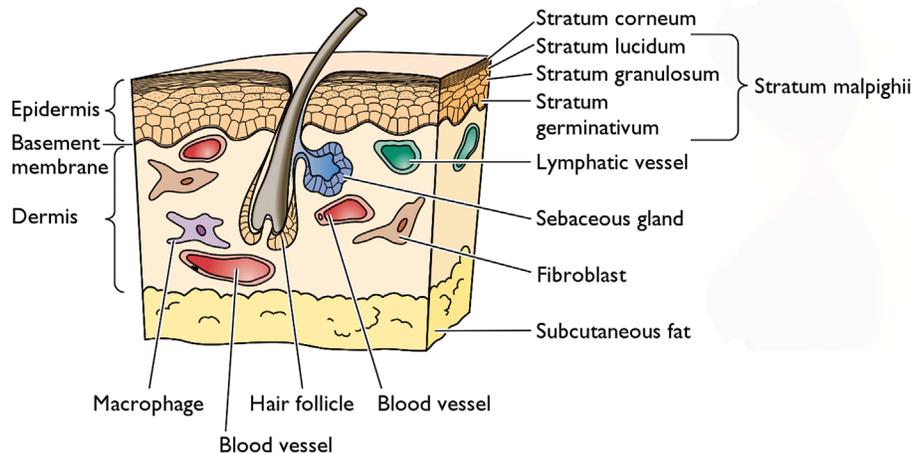


FIGURE 7.3 Cross-section of human skin, showing the different anatomical compartments with their contents. Originally from Fenner, F.J., et al., 1974. *The Biology of Animal Viruses*, Academic Press, New York. Modified from Flint, S.J., et al., *Principles of Virology*, third ed., Vol. II. ASM Press, Washington, DC, with permission.

the infant to disease in later life. There are well-studied examples of this in animals, and the human genome contains large tracts of integrated retrovirus DNA (endogenous retrovirus genomic DNA and fragments of such, making up about 8% of the human genome). However, no human diseases have so far been shown to be transmitted in this way.

2. Transplacental spread, where infection is passed from an infected mother to her fetus during pregnancy—this usually involves infection of the placenta. Outcomes may range from inapparent infection, development of disease in postnatal life, congenital malformations at birth, or premature labor with or without stillbirth. Well-known examples are rubella (congenital rubella syndrome, now well-controlled by vaccination in most developed countries), cytomegalovirus disease (a remaining major infective cause of congenital abnormalities) and now Zika virus encephalitis and microcephaly. With other infections such as HIV and hepatitis B, both transplacental and perinatal transmission can occur.
3. Perinatal transmission resulting from contact with infected genital secretions or bowel contents during delivery. Infection with herpes simplex viruses 1 and 2 and Coxsackieviruses acquired in this way can have severe consequences, more severe than the usual outcome of these infections in later life due to the immaturity of the newborn infant's immune system.

MECHANISMS OF VIRUS SPREAD WITHIN THE BODY

Virus replication may be localized to a body surface or, alternatively, it may become generalized or systemic following spread from entry sites via lymphatic and hematogenous routes.

Local Spread of Virus on Epithelial Surfaces

Viruses that enter the body via the respiratory or intestinal tracts can be spread rapidly through the layer of fluid/mucus that covers epithelial surfaces; consequently such infections often progress rapidly. Spread to more distant parts of the same anatomical space, for example, the sinuses, middle ear, or alveoli in the case of respiratory infections, is enhanced by sneezing, coughing, and inhalation of secretions. Infections of the respiratory tract by paramyxoviruses and influenza viruses and of the intestinal tract by rotaviruses produce little or no invasion of sub-epithelial tissues. Although these viruses usually enter lymphatics and thus have the potential to spread, they usually do not replicate well in deeper tissues.

In the skin, papillomaviruses initiate infection in the basal layer of the epidermis, but maturation of virions occurs only in cells as they move toward the skin surface and become keratinized. Since this is a slow process taking several weeks, papillomas develop slowly. Many poxviruses produce infection via the skin, but in addition to spreading from cell to cell, there is in addition localized sub-epithelial and lymphatic spread. In vaccination with vaccinia virus a few epidermal cells are infected by scarification and virus spreads locally from cell to cell, primarily in the epidermis, before spreading to the local lymph nodes. The poxviruses that cause molluscum contagiosum, orf, and tanapox remain localized in the skin and produce local lesions.

Factors that restrict an infection from spreading beyond an epithelial surface are listed in [Table 7.4](#). One important factor is the directional shedding of viruses from infected polarized epithelial surfaces. If a virus is preferentially released from the apical end of cells either into the lumen of the respiratory or intestinal tracts or the acinar lumen of a gland, it is free to spread locally to contiguous epithelial

TABLE 7.4 Factors that Restrict Virus Spread from an Epithelial Surface

1. Directional (polarized) budding. When infecting a polarized epithelium, some viruses bud preferentially from the *apical* surface toward the lumen, while others bud from the *baso-lateral* surface toward the underlying tissues
2. Virus may be unable to cross the basement membrane unless it is damaged
3. Cell types in more distant parts of the body may lack receptors, or not be permissive for other reasons
4. There may be systemic presence of neutralizing antibody
5. The particular virus strain may be temperature-sensitive, i.e., it may grow successfully in the nasal passages at 33° but not deeper in the body at 37°
6. A fusion protein on the virion may require proteolytic cleavage for its activation; proteases capable of performing this cleavage may be restricted to a particular site, e.g., gastrointestinal or respiratory tract

surfaces and may be immediately shed from the body, but this site does not favor invasion of sub-epithelial tissues and systemic spread. Conversely, shedding from the basolateral cell surfaces of epithelial cells facilitates invasion of sub-epithelial tissues and subsequent dissemination of virus through lymphatics, blood vessels, or nerves. Paramyxoviruses, respiratory syncytial virus, and influenza viruses are released preferentially from luminal (apical) surfaces of respiratory system epithelial cells, whereas HIV 1 and 2 are shed from basolateral surfaces of genital tract and other mucosae into sub-epithelial spaces. The polarized distribution of proteins and lipids at different regions of the cell surface is maintained by the interaction between distinct apical and basolateral sorting signals and the appropriate sorting machineries and transport carriers. Interaction between precursor virus particles and these processes facilitates the transport of viruses from sites of synthesis/assembly to their respective plasma membrane domains along the cell cytoskeleton, microtubules, and other framework constituents. Tight junctions at cell-to-cell contact points prevent the movement of proteins between the two domains and maintain the unique protein composition of each domain. In influenza and respiratory syncytial virus-infected epithelial cells, the viral ribonucleoprotein complex (RNP) is carried to a plasma membrane domain where at the same time viral surface glycoproteins are inserted into lipid rafts in the plasma membrane at the nascent budding site. Specific signaling sequences in the stem of the viral glycoprotein spikes are involved along with M-protein determinants. Much of the specificity in the pattern of spread of viruses through the host is due to such evolutionary processes.

Infections that are restricted to an epithelial surface are not always associated with less severe clinical disease.

Large areas of intestinal epithelium may be damaged by rotaviruses, causing severe diarrhea and even death from dehydration. The severity of localized infections of the respiratory tract depends upon their location; infections of the upper respiratory tract may produce severe rhinitis but few other signs; tracheitis lesions are usually repaired rapidly; infection of the bronchioles or alveoli more often produce severe respiratory distress. The entire tracheal and bronchial epithelial lining may be destroyed in influenza or parainfluenza virus infections, causing extravasation of fluids and hypoxia. Fluid and cellular debris build-up in airways may predispose to anoxia and secondary bacterial invasion.

To this point, examples have been considered where infection is typically confined to the epithelial surface of the initial entry site. Of course, in some cases, for example, in immunosuppressed individuals, such infections may become generalized. Furthermore, some superficial infections, for example, influenza, can lead to systemic symptoms including headache, malaise, and myalgia, due to the action of circulating cytokines (the extreme being called a “cytokine storm”)—inflammatory mediators may act systemically even if the virus itself remains localized.

Mechanisms of Virus Spread to Distant Target Organs

The most important routes for dissemination of virus to target organs are via blood capillaries, within macrophages and dendritic cells, or via lymphatics. Circulating virus may then gain access to tissues by one of a number of mechanisms, depending on the type of blood–tissue junction present. Another mechanism for virus dissemination is where virus enters local nerve endings and undergoes retrograde transit within nerve axons (see below).

Sub-Epithelial Viral Invasion and the Network of Lymphatics

Viruses can enter the network of lymphatics beneath all cutaneous and mucosal epithelia (Fig. 7.4). Virions entering the lymphatics are carried to local draining lymph nodes. As they enter the marginal sinuses of lymph nodes, virus particles are exposed to active macrophages and dendritic cells and may be engulfed, inactivated, and/or processed for viral antigen presentation to adjacent lymphocytes, thereby initiating the adaptive immune response (see Chapter 6: Adaptive Immune Responses to Infection). Some viruses, however, also replicate in monocytes/macrophages (e.g., many retroviruses, measles virus, dengue viruses, some adenoviruses, and some herpesviruses); some viruses are able to replicate in lymphocytes. Some viruses pass directly through lymph nodes to enter the bloodstream. Monocytes and lymphocytes recirculate through the blood, lymphatics,

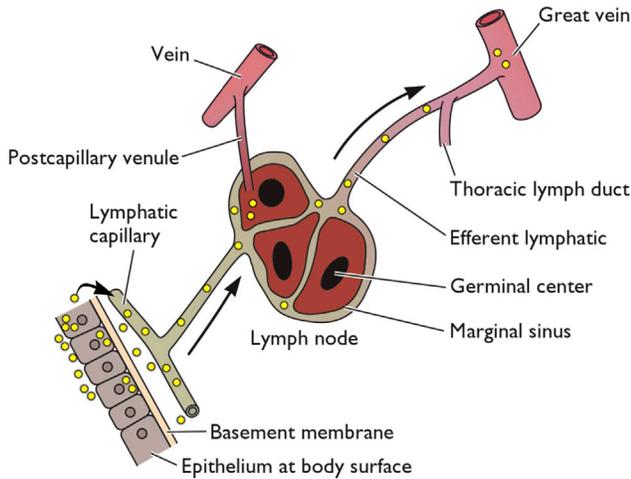


FIGURE 7.4 Subepithelial invasion and lymphatic spread of viruses. Originally from the work of Cedric Mims 1977–2015 (e.g., *Pathogenesis of Infectious Disease*, through six editions, Elsevier/Academic Press), and modified many times since.

and lymph nodes; recirculation is the key to normal “immune surveillance,” but is also an effective means of disseminating some viruses throughout the body.

Normally, there is a local inflammatory response at the site of viral invasion, the extent of which depends upon the extent of tissue damage. Local blood vessels become dilated and rendered more permeable, so that monocytes and lymphocytes, cytokines, immunoglobulins, and complement components may be delivered directly into the extravascular site of infection. These events are especially vigorous once the adaptive immune response reaches its full level of activity. In some cases, viruses take advantage of these events to infect cells of the lymphoreticular system and spread locally or systemically.

Virus Spread via the Bloodstream: Viremia

The blood is the most effective and rapid vehicle for the spread of viruses through the body. Once a virus has reached the bloodstream, usually via the lymphatic system, it can localize in any part of the body within minutes. The first entry of virus into the bloodstream is referred to as the *primary viremia*. This early viremia may be clinically silent, known to have taken place only because of the invasion of distant organs. Virus replication in initial target organs leads to the sustained production of much higher concentrations of virus, producing a *secondary viremia* (Fig. 7.5), which in turn can lead to the establishment of infection in yet other parts of the body.

Virions may be free in the plasma or may be contained in, or adsorbed to, leukocytes, platelets, or erythrocytes. Enteroviruses, togaviruses, and most flaviviruses circulate free in the plasma, whereas hepatitis B and hepatitis C

viruses are complexed with different serum proteins and lipids. Viruses carried in leukocytes, generally lymphocytes or monocytes, are not cleared as readily or in the same way as viruses circulating free in the plasma; being protected from antibodies and other plasma components, virus can be transported to distant tissues even after the initiation of the immune response. Monocyte-associated viremia is a feature of measles, cytomegalovirus, and human herpesvirus 8 (Kaposi sarcoma herpesvirus) infections. In infections caused by Rift Valley fever virus and Colorado tick fever virus, virions are associated with erythrocytes—in Colorado tick fever virus infection the virus replicates in erythrocyte precursors in the bone marrow producing a viremia that lasts for the life span of erythrocytes. Colorado tick fever virus has been transmitted to blood transfusion recipients 100 days after the donor was infected by tick bite. Certain murine leukemia viruses and arenaviruses infect megakaryocytes and thereby are present in circulating platelets, the clinical significance of which is unknown. Neutrophils have a very short life span and powerful antimicrobial mechanisms; they are rarely infected despite frequently containing phagocytosed virions.

Viruses circulating in the blood encounter many kinds of cells, but two play special roles in determining the subsequent fate of infection: macrophages and vascular endothelial cells.

Virus Interactions with Macrophages

Macrophages are very efficient phagocytes and are present in all compartments of the body: they occur free in plasma, in alveoli, in sub-epithelial tissues, in sinusoids of the lymph nodes, and above all in the sinusoids of the liver, spleen, and bone marrow. Together with dendritic cells and B lymphocytes, macrophages are antigen-processing and antigen-presenting cells and therefore play a pivotal role in initiation of the adaptive immune response (see Chapter 6: Adaptive Immune Responses to Infection). The antiviral action of macrophages depends on the age and physiological status of the host and their site of origin in the body; indeed, even in a given site there are subpopulations of macrophages that differ both in phagocytic competence and in susceptibility to infection. Their state of activation is also important. Transport of viruses inside infected cells such as macrophages, has been referred to as the “*Trojan Horse*” mechanism of invasion; it is especially important in HIV infection of the central nervous system.

The various kinds of interactions that can occur between macrophages and viruses may be illustrated by the various responses of Kupffer cells, the macrophages lining the sinusoids of the liver (Fig. 7.6). Infection of Kupffer cells often contributes to acute hepatocellular damage (e.g., yellow fever, Rift Valley fever, Crimean Congo hemorrhagic fever, and Ebola and Marburg virus hemorrhagic fevers).

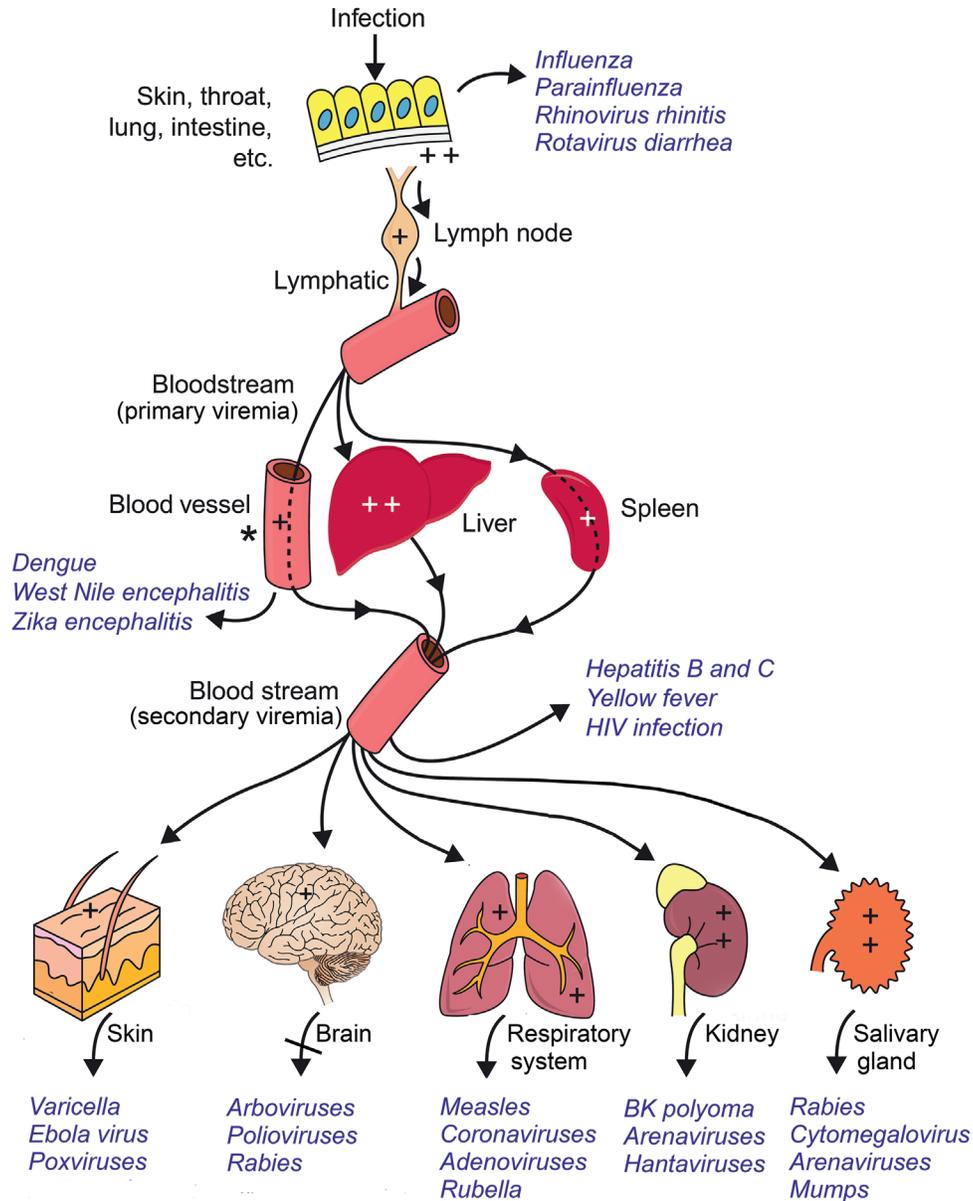


FIGURE 7.5 The role of primary and secondary viremias in the spread of viruses throughout the body, showing some, but not all, sites of replication and routes of shedding. + indicates major sites of virus replication; arrows indicate sites of shedding to the exterior; * indicates replication in vascular endothelium rather than in viscera. Originally from the work of Cedric Mims, 1977–2015. (e.g., *Pathogenesis of Infectious Disease*, through six editions), Elsevier/Academic Press), and modified many times since.

Differences in virus–macrophage interactions may account for differences in the virulence of particular virus strains and differences in host resistance. Even though macrophages are innately efficient phagocytes, this capacity is greatly enhanced during an immune response by the action of cytokines, which are released notably from T helper lymphocytes. Macrophages also have Fc-receptors and C3-receptors on their plasma membranes, which further enhance the ingestion of virus particles, especially when coated with antibodies or complement. Certain togaviruses, flaviviruses (notably dengue viruses), coronaviruses, arenaviruses, reoviruses, and especially

retroviruses, are capable of replicating in macrophages—when virus uptake is facilitated by bound antibody, antibody-mediated enhancement of infection may occur. This is a major pathogenic factor in dengue and retrovirus infections.

Virus Interactions with Vascular Endothelial Cells

The vascular endothelium with its basement membrane and tight cell junctions constitutes the blood–tissue interface and is often a barrier. Virus invasion of the tissue parenchyma

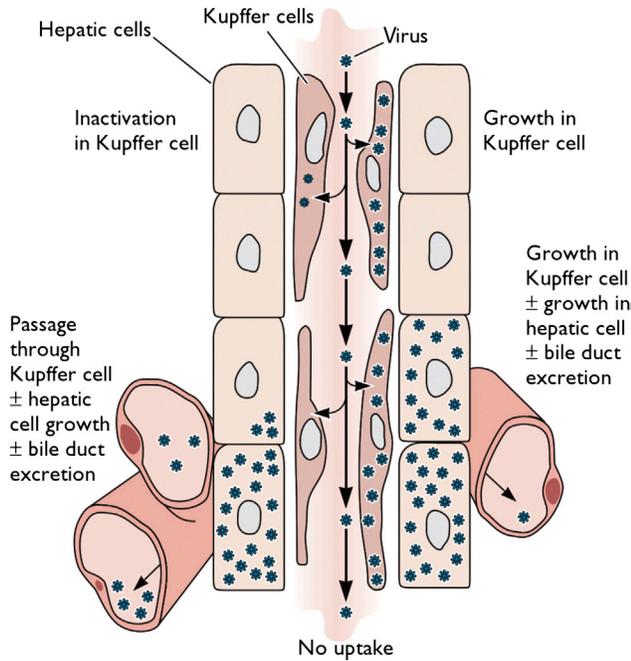


FIGURE 7.6 Different possible interactions between viruses and Kupffer cells, the macrophages that line the sinusoids of the liver. (1) Viruses may pass through the sinusoid without being phagocytosed. (2) Virions may be phagocytosed and destroyed: because the macrophage system is so efficient, viremia can only be maintained if virions enter the blood stream as fast as they are removed. (3) Virions may be phagocytosed and then transferred passively to adjacent cells (i.e., hepatocytes in the liver). (4) Virions may be phagocytosed by Kupffer cells and then replicate in them, with or without spread to adjacent hepatocytes and excretion into bile ducts or release to the bloodstream. Originally from the work of Cedric Mims, 1977–2015 (e.g., *Pathogenesis of Infectious Disease, through six editions*), Elsevier/Academic Press), and modified many times since.

by circulating virions depends upon crossing this barrier, usually exiting capillaries and venules where the blood flow is slower and the barrier is thinnest. The structure of this barrier varies from tissue to tissue (Fig. 7.7): (1) in the central nervous system, connective tissue, muscle, skin, and lungs there is a continuous lining of endothelium and basement membrane, (2) in the intestine, renal glomerulus, pancreas, endocrine glands, and choroid plexus, this lining has fenestrations or pores, (3) in liver, spleen, bone marrow, and adrenal glands, blood flows through sinusoids lined by macrophages and endothelial cells. Virions may either move passively through fenestrae between endothelial cells, or replicate in endothelial cells or macrophages and “grow” across this barrier, (4) in some cases viruses can be passively transferred through the lining cells without replicating (“transcytosis”), or (5) be carried within lymphocytes or monocytes trafficking between lining cells (“diapedesis”).

The above mechanisms do not fully explain the preferential targeting of certain viruses to particular target tissues, which is likely to involve use of preferred mechanisms to exit from the vascular space in preferred organs, as well as a preference for replication in particular cell types. Much focus has been placed upon viral receptors on particular cell types, but the identification of virus receptors in cultured cells has turned out to oversimplify this complex subject. A virus may employ several different receptors and co-receptors *in vivo*, on endothelial cells, perivascular cells, parenchymal cells, etc., and these may vary between different target organs. While our knowledge of virus/receptor interactions has advanced greatly in recent years, our understanding of the full mechanisms underlying virus dissemination and tissue targeting *in vivo* is still limited.

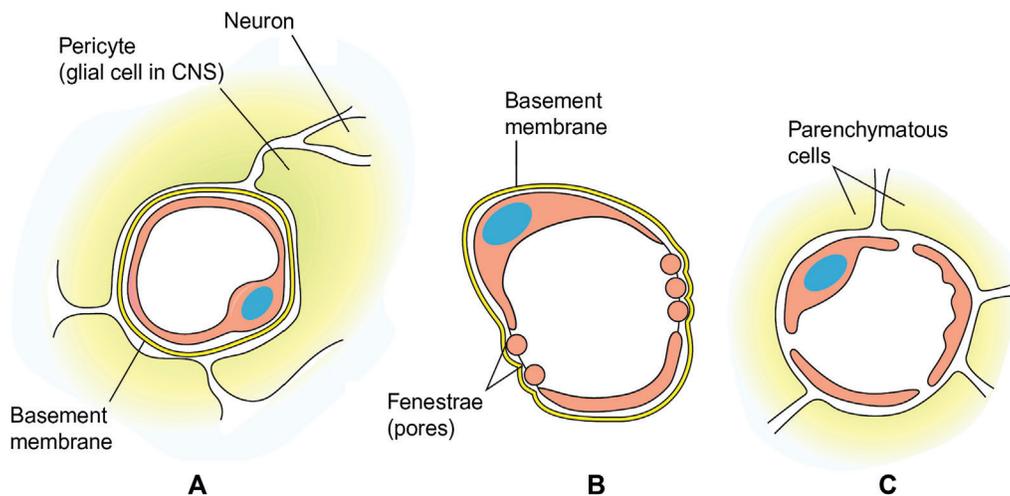


FIGURE 7.7 Three types of blood–tissue junctions, as occur in capillaries, venules, and sinusoids. (A) Continuous endothelium (central nervous system, connective tissue, skeletal and cardiac muscle, skin, lung). (B) Fenestrated endothelium (renal glomerulus, intestinal villi, choroid plexus, pancreas, endocrine glands). (C) Sinusoid (reticuloendothelial system): liver (lined by Kupffer cells), spleen, bone marrow, adrenal gland, parathyroid gland. Originally from the work of Cedric Mims, 1977–2015 (e.g., *Pathogenesis of Infectious Disease, through six editions*), Elsevier/Academic Press), and modified many times since.

Maintenance of Viremia

Viremia can be maintained only if there is a continuing introduction of virus into the bloodstream from infected tissues to counter the continual removal of virus by macrophages and other cells and the natural decay of virus infectivity over time. The balance between virus replication and removal is greatly affected if macrophage functions are impaired, as in some infections (e.g., measles). Although circulating leukocytes can themselves constitute a site of viral replication, viremia is usually maintained by replication in parenchymal cells of target organs such as the liver, spleen, lymph nodes, bone marrow, etc. In some infections, such as dengue, viremia follows infection of endothelial cells. Striated and smooth muscle cells may be an important site of replication of some enteroviruses, togaviruses, and rhabdoviruses, from where virus reaches the blood via lymphatic circulation/recirculation.

There is generally a correlation between the magnitude of viremia generated by blood-borne viruses and their capacity to invade target tissues. Conversely, the failure of some attenuated viruses to generate a significant viremia may account for their lack of invasiveness. Certain neurotropic viruses are virulent after intracerebral inoculation, but avirulent when inoculated peripherally—in such instances the level of viremia may be insufficient to favor invasion of the nervous system, or this may be due to other virus characteristics. However, the capacity to produce viremia and the capacity to invade tissues from the bloodstream are distinct viral properties. For example, some laboratory strains of Semliki Forest virus, an alphavirus, have lost their capacity to invade the central nervous system while retaining a capacity to generate a viremia equivalent in duration and magnitude to that produced by neuroinvasive strains.

In most transient systemic infections, the viremic phase is usually quite short-lived, typically a few days. As this often coincides with the period when the patient feels most unwell, and is therefore excluded from donating blood, transmission of these infections by blood transfusion or sharing of intravenous needles is often not a significant problem. One notable exception is hepatitis A; in this case there is a transient infection, but low levels of infectious virus can circulate for several months, into convalescence, and be transmitted by the blood-borne route. This situation is however quite different from the persistent systemic infections discussed below, where high-level viremia can be maintained for months or years frequently without symptoms. Transmission of these infections by unscreened donated blood can present major problems (e.g., hepatitis B virus, HIV).

Virus Spread via Nerves

Herpesvirus capsids travel centripetally from the peripheral nervous system entry site to the central nervous system in the

axon cytoplasm of sensory nerves; while doing so they also sequentially infect the Schwann cells of the nerve sheath. In most instances herpes simplex and varicella-zoster viruses stop at this point, establishing persistent infection of the neurons of dorsal root ganglia. These viruses can then transit the same sensory nerves centrifugally from the ganglia to the skin/mucosa. This is what happens in the reactivation of latent infection and production of recrudescing epithelial lesions. The rate of this anterograde and retrograde transit of virus nucleocapsids approaches 200 to 400 mm a day.

Rabies virus travels to the central nervous system within the axon cytoplasm without infecting cells of the nerve sheath (Schwann cells). Following the bite of a rabid animal, virus is usually amplified by replication in striated muscle cells at the bite site. From here virus may enter the peripheral nervous system at sensory nerve end organs in muscle (neuromuscular spindles) or motor nerve end organs (motor end plates), traveling in axon cytoplasm centripetally. Rabies virus may also enter the peripheral nervous system end organs directly at the bite site: this is the likely situation in rabies cases with an exceptionally short incubation period. Rarely, the central nervous system may be invaded directly by rabies virus and some togaviruses when neurons of the olfactory end organ in the nares are directly exposed. These are the only neurons in the body that directly link the body surface with the central nervous system. In instances where virus transit uses this route, the olfactory bulb of the brain is usually seen to be infected first. This has been the likely source of infection in speleologists infected in bat caves where the rabies virus has been shown to be present in aerosols (in some areas speleologists are regularly vaccinated).

As these viruses move centripetally, they must cross cell-to-cell junctions. Rabies virus and herpesviruses are known to cross from one neuron to the next at synaptic junctions by employing structures and mechanisms normally used to transfer neurotransmitter molecules.

VIRUS INFECTION OF TARGET ORGANS

Different viruses present different unique patterns of infection (clinical signs, symptoms, laboratory data, etc.), based upon differences in their major sites of replication and damage. These sites are known as “target organs.” Of course, other sites may also be infected at various stages of infection, without necessarily being clinically evident ([Table 7.5](#)).

The predilection of a particular virus for infecting a particular cell type or organ is known as its “tropism” (as distinct from “trophism,” which refers to nutrients). With some viruses, the pathophysiological, molecular, and/or anatomical factors determining tropism have been partially clarified, but for many others these are not known. For example: most viruses of humans replicate optimally at 37°C, the internal temperature of the body. However some

TABLE 7.5 Target Organs in Some Acute Transient Systemic Infections

Virus enters via the gastrointestinal tract	
•	Hepatitis A: liver
•	Poliomyelitis: anterior horn cell in spinal cord and central nervous system
•	Other enteroviruses: meninges, muscle, skin, CNS
Virus enters via the respiratory tract	
•	Chickenpox: (sensory ganglia), skin
•	Measles: conjunctiva, skin, CNS
•	Rubella: skin, joints
•	Mumps: parotid and salivary glands, testes, pancreas, meninges
•	Smallpox: skin, mucous membranes

TABLE 7.6 Virus and Host Properties Affecting Viral Tropism

1. Distribution of virus receptors may be restricted to particular cells or expressed only at certain times or under certain physiological conditions
2. Temperature sensitivity of virus replication
3. Restricted presence of a specific cellular protease required for activation of virus attachment or fusion proteins
4. Selective presence of host innate or adaptive immune response
5. Cellular transcription factors restricted to specific cell types
6. Anatomical barriers may restrict virus spread
7. Route of inoculation or entry may dictate virus distribution

respiratory viruses, for example, rhinoviruses, replicate optimally at 33°C, reflecting the slightly lower temperature of the mucosal surfaces of the upper respiratory tract. These viruses grow less well at 37°, which restricts spread and involvement of the lower respiratory tract. This also means that rhinovirus isolation is more successful if cell cultures are maintained at 33°. Many other factors also affect virus infection patterns (Table 7.6).

Of course, the summary items listed in Table 7.6 represent necessary simplifications. For example, the poliovirus receptor (Pvr, CD155), a member of the immunoglobulin (Ig) superfamily of proteins, is present on neurons in many parts of the nervous system as well as on cells of the adrenal gland, lung, and kidney, and at low levels on skeletal muscle. Poliovirus replication in the infected host partly follows this distribution but there are a number of discordances, indicating that receptor distribution alone is not sufficient to explain the differences in virus distribution. Most striking is the selective infection and rapid destruction by polioviruses of anterior horn neurons of the spinal cord, while other neurons are often spared.

Furthermore, not only do some viruses require several cellular receptors/co-receptors to complete an infection cycle, some utilize different receptors on different host cells

in different organs or tissues. For example, the glycoprotein ligand SU of HIV-1 can bind to several receptors (including CD4, CXCR4, CCR5, and others); these take part in a complex sequence of initial loose binding, tight binding, fusion, and entry of the viral RNA complex into the cell cytosol. HIV-1 can infect T lymphocytes, macrophages, and other cell types; viral strains that bind CXCR4 preferentially infect T cell lines, and strains that bind CCR5 infect monocytes/macrophages, but the full role of different receptor usage by different HIV variants in natural infection remains to be clarified. Expression of receptors can be dynamic; for example, it has been shown experimentally that animals treated with neuraminidase [the viral enzyme that digests the neuraminic acid (NA)-containing receptor] exhibit substantial protection against intranasal infection with influenza viruses, and this lasts until the neuraminidase-sensitive receptors have regenerated.

Receptors for a particular virus are usually restricted to certain cell types in certain organs, and only these cells can be infected. However, the presence of critical receptors is not the only factor that determines whether the cell may become infected—intracellular factors that exert effects subsequent to virus attachment, such as viral enhancers and cellular factors, are also required for a productive infection.

The Skin as a Target Organ

As well as being a site of initial virus entry, the skin may be invaded via the bloodstream, producing erythema and often a generalized rash. The individual lesions in a generalized rash may progress sequentially through macules, papules, vesicles, pustules, and ulcers, or alternatively the rash may resolve at an earlier stage. A lasting local dilation of subpapillary dermal blood vessels produces a *macule*, which becomes a *papule* if there is also edema and infiltration of inflammatory cells into the area. Primary involvement of the epidermis or separation of epidermis from dermis by fluid pressure leads to *vesicle* formation, characterized by the presence of clear fluid. This may subsequently be converted to a *pustule* by polymorphonuclear cell infiltration into the vesicle. Erosion or sloughing of the surface epithelium produces an *ulcer*, with or without *scab* formation. More severe involvement of the dermal vessels may lead to petechial or hemorrhagic rashes, although coagulation defects and thrombocytopenia may also be important in the genesis of such lesions. The clinical distribution, stage of evolution, and other characteristics of rashes can be distinctive for specific viruses and an aid in diagnosis (Fig. 7.8).

Virus Infection of the Central Nervous System

Because of the critical importance of the central nervous system and its vulnerability to damage by any process

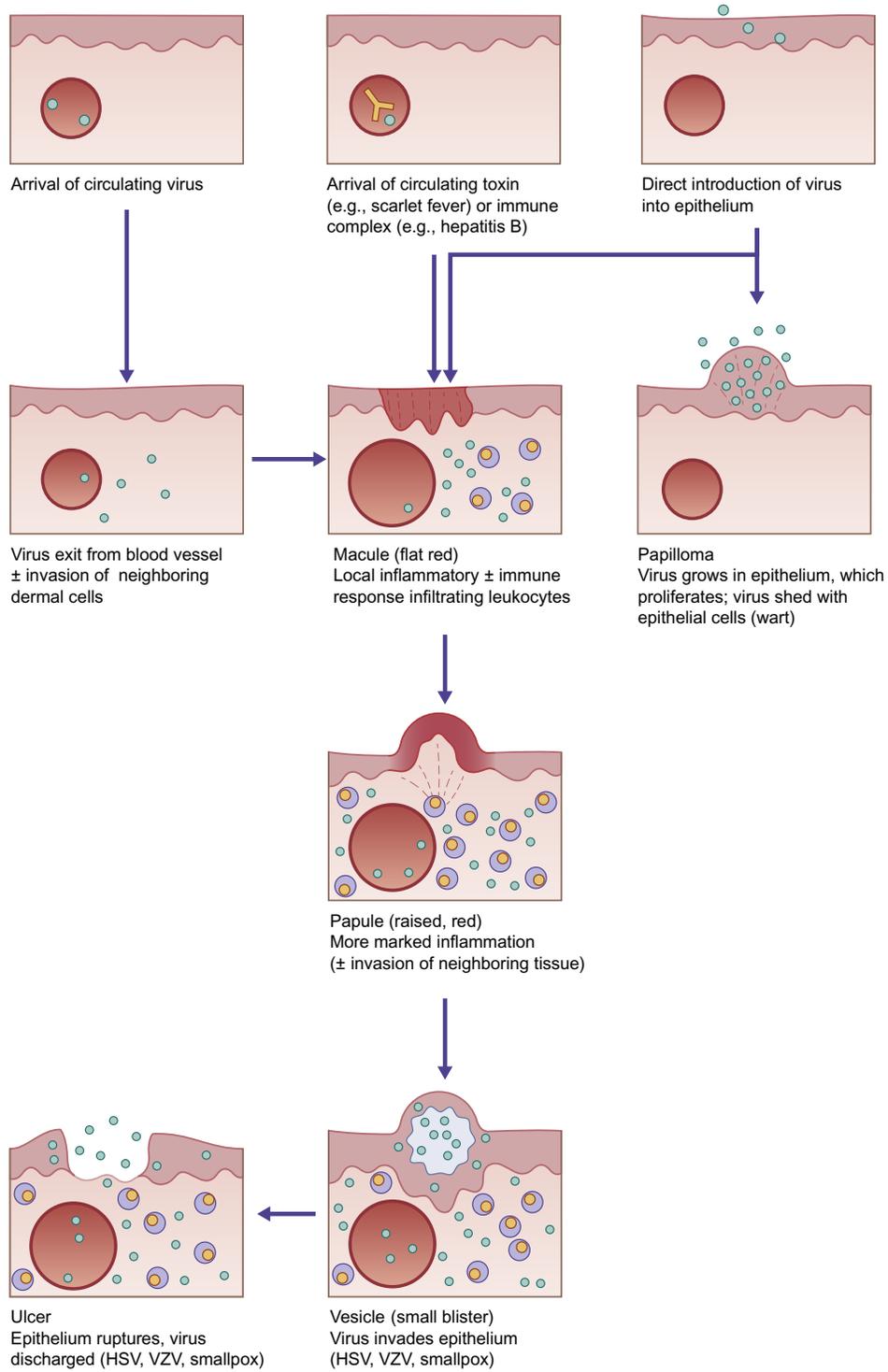


FIGURE 7.8 Sequential stages in the evolution of skin rashes. In some virus infections, the rash may not progress beyond the maculopapular stage (e.g., rubella), while with others a full progression through to vesicles and ulcers is usual (e.g., smallpox). *Modified from Mims, et al., 1993. Medical Microbiology, Mosby/Elsevier Europe Ltd.*

that harms neurons directly or via increased intracranial pressure, viral invasion of the central nervous system is always of serious concern.

Viruses can spread from the blood to the brain in two major ways:

- A. Virus present within blood vessels in the meninges and the choroid plexus can pass through the endothelial lining (with or without replication) to circulate in the cerebrospinal fluid, infect the ependymal lining of the ventricles, and thence invade the brain parenchyma (Fig. 7.9). Some enteroviruses that cause meningitis, rather than encephalitis, may traverse endothelial junctions in the meninges and stay localized at this site without further penetration of the brain parenchyma. Others progress from this site to cause encephalitis.
- B. Although the cerebral capillaries together with their underlying dense basement membranes represent a

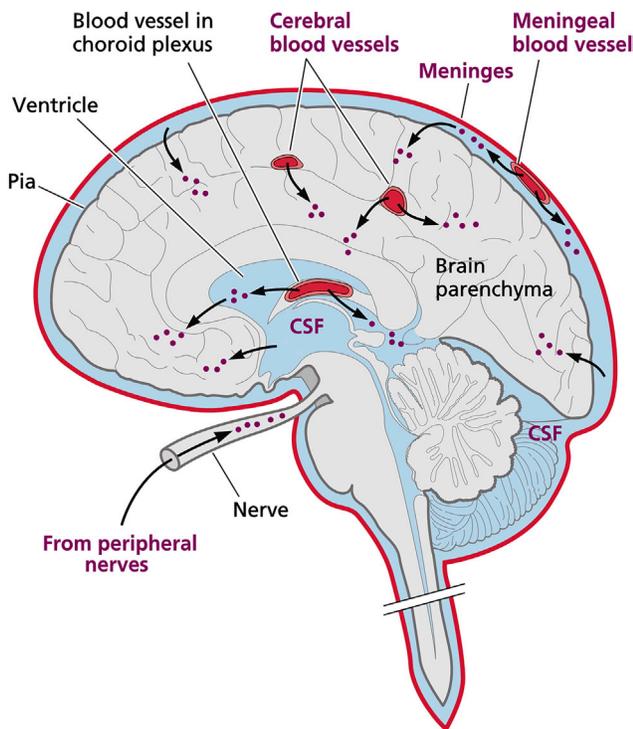


FIGURE 7.9 Viruses can gain access to the central nervous system via many routes, with specific routes seen in certain experimental models; however, in most human central nervous system diseases caused by viruses the route of entry is unknown or based upon indirect evidence. Few, such as rabies virus and herpesviruses, ascend via peripheral nerves; most employ hematogenous routes, entering via the capillaries of the brain parenchyma or surfaces (meninges, ventricles, choroid plexus). CSF, cerebrospinal fluid. Originally from Mims, C.A., 1977–2008. *Pathogenesis of Infectious Disease (through five editions)*. Elsevier/Academic Press, London, UK. Then adapted by Nathanson, N. (ed.), 1997. *Viral Pathogenesis*. Lippincott-Raven, Philadelphia, PA. Then adapted by Flint, S.J., et al., 2009. *Principles of Virology: Pathogenesis and Control*, 3rd ed. ASM Press, Washington, DC.

morphological blood–brain barrier, most viruses that invade the central nervous system cross these vessel walls directly. Some viruses infect vascular endothelial cells prior to infection of the cells of the brain parenchyma; others appear to be transported across the capillary walls without endothelial cell infection. In HIV infection and measles, virus may be carried across capillary walls into the brain parenchyma via infected leukocytes. Subsequent spread in the central nervous system can take place via the cerebrospinal fluid or by sequential infection of neural cells. The intercellular space of the brain parenchyma may appear completely collapsed and a barrier to virus movement, but in fact it is without barriers (e.g., tight junctions, connective tissue structures) and so local virus movement between neurons, glia, and inflammatory cells occurs causing focal parenchymal lesions.

The other important route of invasion of the central nervous system is via the peripheral nerves, as seen in rabies and varicella-zoster and herpes simplex encephalitis. This route has been described in the preceding section.

Lytic infections of neurons, whether due to polioviruses, flaviviruses, togaviruses, or herpesviruses, are characterized by the three histological hallmarks of viral encephalitis: (1) neuronal necrosis, (2) phagocytosis of neurons by phagocytic cells (neuronophagia), and (3) perivascular infiltration of mononuclear cells (perivascular cuffing), the latter reflecting an immune response. The cause of clinical neurological signs in many central nervous system infections is sometimes less clear. Rabies virus (“street virus” = wild-type strains) infection is rather non-cytocidal; infection evokes little of the inflammatory reaction or cell necrosis found in other encephalitides, yet it is lethal for most mammalian species. The basis for this is not clear but has to do with pathophysiologic functional loss. The extensive central nervous system infection of mice congenitally infected with non-cytolytic lymphocytic choriomeningitis virus, readily demonstrable by fluorescent antibody staining, has no recognizable deleterious effect—it is the following fulminant T lymphocyte response that is lethal (immunopathological disease). Characteristic pathological changes are seen in several slowly progressive diseases of the central nervous system; in prion diseases, for example, there is slow neuronal degeneration and vacuolization (Chapter 38: Prion Diseases); in progressive multifocal leucoencephalopathy (PML), JC virus infection is lytic in oligodendrocytes, producing multiple foci of demyelination and bizarre transformation of astrocytes into giant cells (Chapter 20: Polyomaviruses).

Post-infectious encephalitis occurs most frequently after measles (about 1 per 1000 cases), more rarely after rubella and varicella-zoster virus infections, and in the past was seen in 1 to 2 persons per 100,000 primary vaccinations against smallpox (see below).

Virus Infection of Other Organs

Almost any organ may be infected via the bloodstream with many viruses, but most viruses have well-defined organ and tissue tropisms. The clinical importance of infection of various organs and tissues depends in part on their role in the economy of the body. Thus invasion of the liver, causing severe hepatitis, as in yellow fever and infections with the hepatitis viruses, may be life-threatening, with the additional possibility in the case of hepatitis B and C of the establishment of a chronic carrier state that may eventually result in hepatocellular carcinoma. The critical importance of such organs as the brain, heart, and lung is equally self-evident. Thus the most dangerous viral infections tend to be those that cause encephalitis, pneumonia, carditis, hepatitis, or hemorrhagic fever.

Infection of the testis or accessory sexual organs may lead to excretion of virus in the semen and the risk of transmission during sexual activities. In some cases of hemorrhagic fever, virus continues to be shed in the semen long into convalescence. Localization of virus in the salivary glands (e.g., in mumps), mammary glands, kidney tubules, and lungs may lead to excretion in the saliva, milk, urine, and respiratory secretions. Some togaviruses and Coxsackieviruses infect muscle cells, while infection of synovial cells by rubella virus, Ross River virus, and chikungunya virus produces polyarthritis/polyarthralgia.

Virus Infection of the Fetus

Most viral infections of the mother have no harmful effect on the fetus, but some blood-borne viruses cross the placenta to enter the fetal circulation, sometimes after establishing foci of infection in the placenta. Severe cytolytic infections of the fetus cause fetal death and abortion, a pattern that was common in smallpox. Fetal death and nearly universal death of the mother is the case in Ebola, Marburg, and Lassa hemorrhagic fevers. Fetal teratogenic effects are common following rubella and cytomegalovirus infections during pregnancy.

Since Norman Gregg's initial observations in 1941, it has been recognized that maternal rubella contracted in the early months of pregnancy often leads to congenital abnormalities. A variety of abnormalities occur, of which the most severe are deafness, blindness, and congenital heart and brain defects. These defects may not be recognized until after the birth of an apparently healthy baby, or they may be associated with severe neonatal disease—hepatosplenomegaly, purpura, and jaundice—comprising the “*congenital rubella syndrome*.” The pathogenesis involves infection of the placenta from maternal viremia, leading to virus spread through the developing vasculature of the fetus. Damage to fetal blood vessels and ischemia in affected organs cause a pattern of congenital defects, that are typically most prominent in those particular organs that are being formed at the gestational time when the

mother acquires her infection. Classical immunological tolerance does not develop; children who have contracted rubella *in utero* exhibit high titers of neutralizing antibodies throughout their lives but there may be some diminution in cell-mediated immunity. Rubella virus is relatively non-cytocidal; few inflammatory or necrotic changes are found in infected fetuses. The retarded growth and developmental abnormalities may be due, in addition to ischemic effects, to slowing of cell division leading to the reduced numbers of cells in many fetal organs. Clones of persistently infected cells might be unaffected by the maternal antibody that develops during the first weeks after the maternal infection, even though such antibody may limit viremia in the fetus, perhaps a matter of “too little, too late.”

Cytomegalic inclusion disease of the newborn results from infection acquired congenitally from mothers suffering an inapparent cytomegalovirus infection during pregnancy. The important clinical features in neonates include hepatosplenomegaly, thrombocytopenic purpura, hepatitis and jaundice, microcephaly, and mental retardation.

Apart from infection of the fetus via the placenta, germ-line transmission of retroviruses, as integrated provirus, occurs commonly in many species of animals but has not been found to be linked to disease in humans. More important in human medicine because of its epidemiological implications (see Chapter 14: Control, Prevention, and Eradication) is transovarial transmission of arboviruses, notably some bunyaviruses, togaviruses, and flaviviruses, in mosquitoes and ticks. This mechanism may be involved in virus overwintering in vector species leading to a long-term risk of human infection in certain ecoiniches.

PERSISTENT VIRUS INFECTIONS— MECHANISMS INFLUENCING PERSISTENCE

The great majority of virus infections of humans are transient, and after a period of replication within the body and development of an immune response (with or without accompanying pathology and symptoms) the virus is eliminated from the body. In some instances what is thought to be a persistent viral infection may actually be a chronic bacterial super-infection; for example, sinusitis or bronchitis following a viral infection. In other instances, apparently long-term clinical effects may be a consequence of sequential infections with different viruses. However, more and more viruses that were usually thought only to cause acute transient clinical infections, are being shown to sometimes persist in sequestered sites in trace amounts, usually without any demonstrable effect, but sometimes causing important clinical consequences.

Establishment and maintenance of a persistent infection implies two obvious prerequisites: (1) the failure to

eliminate the virus by the immune system, and (2) in the case of cytotoxic viruses, a restriction of viral replication. The viral genome may persist in the absence of complete cycles of viral replication; persistent infection may only be evident after reactivation of a latent viral state at some later time in the life of the host. Whether persistence leads to disease is obviously of crucial importance to human hosts, but may be of little consequence to virus survival in nature; this depends more critically upon shedding into the environment and consequent spread to other hosts.

Long-term virus persistence is an outcome that is ultimately dependent on the balance between the virus' capacity, on the one hand, to replicate or remain within long-lived cells, and, on the other hand, the virus' ability to counter the two arms of the immune response. The outcome of this balance can vary from situation to situation.

For example, certain viruses, for example, members of the herpesvirus family and HIV, invariably produce persistent infections, whereas other viruses, for example, common respiratory viruses, almost invariably produce a transient infection. Others, for example, hepatitis B virus, can produce either a transient or persistent infection depending on the circumstances, for example, the age of the person at the time of exposure. These "typical" outcomes may also vary subject to the immune competence of the individual—immunosuppression will shift the balance in favor of virus persistence, and in immunosuppressed individuals some typically transient virus infections may persist, and other typically localized or latent infections may become generalized. The following sections describe many of the mechanisms used by different viruses in developing persistence (Table 7.7).

TABLE 7.7 Mechanisms for Ineffective Immune Responses in Persistent Viral Infections^a

Phenomenon	Mechanism	Example ^b
Reduced or absent antigen expression	Limited viral gene expression	Herpes simplex virus (neurons)
		EBV (B lymphocytes)
		HIV-1
Evasion of immune response	Cell–cell spread by cell fusion	HIV-1
		Measles
		Cytomegalovirus
	Sequestration in sanctuaries	HIV-1 (brain)
		CMV, polyoma virus (luminal surface of kidneys, glands)
Antigenic drift	HIV-1, visna, HCV	
Immunosuppression by infection of effector cells	Disruption of lymphocyte function, cell loss. Polyclonal B cell activation. Abrogation of macrophage function	HIV (CD4+ T cells), EB virus, HIV
	Antibody-enhanced infection of macrophage via Fc receptor	Many viruses
Induction of immunologic tolerance	Congenital or neonatal infection induces T cell non-responsiveness	Congenital rubella and CMV, LCM, HBV, HIV, parvovirus B19
Reduced antigen display on plasma membrane	"Stripping" by antibody. Down-regulation of MHC antigen. Down-regulation of cell adhesion molecules Virus-coded Fc receptor blocks immune lysis	Measles (SSPE brain). Adenoviruses. EB virus (Burkitt's lymphoma) Herpesviruses
Blocking by non-neutralizing antibodies		Many viruses
Excess soluble antigen as decoy		Hepatitis B virus
Evasion of cytokines	Interfere with IFN, TNF	Adenoviruses, HCV, others

^aSome of these mechanisms are also operative in non-persistent infections.

^bSpeculative only; in several of these instances an association exists but no cause-and-effect relationship has been demonstrated between the immunologic phenomenon and the persistent infection listed.

Reduction or Absence of Either Viral Gene Expression or Cytocidal Activity

Restricted Expression of Viral Genes

Obviously a virus cannot persist in a cell it destroys. Therefore, long-term persistence of a potentially cytotoxic virus can occur only if the viral genome remains fully or partially silent, or if a dynamic can be established whereby newly made viruses can continue to find new uninfected cells. In latent herpesvirus infections, only a few early genes are transcribed during latency. Latency is eventually overridden, often following immunosuppression and/or by the action of a cytokine or hormone that de-represses transcription of the whole viral genome, leading to reactivation of viral synthesis. In contrast, persistent HIV infection is characterized by continuing virus production in T lymphocytes leading to cell death. This is combined with massive parallel proliferation of uninfected lymphocytes which become a target for further rounds of HIV replication and damage.

Restricted viral genome expression as seen in several persistent infections naturally limits the presentation of viral antigens to the immune system, allowing such infected cells to remain immunologically invisible. For example, in herpes simplex virus infection latently infected neurons display no viral antigens. This protects these cells not only against cytotoxic T cells but also against lysis by antibodies or complement-mediated mechanisms or possibly by antibody-dependent cell-mediated cytotoxicity (ADCC).

Latency in Non-Permissive, Resting, or Undifferentiated Cells

A given virus may undergo productive replication in one cell type but non-productive latent infection of another. For example, Epstein Barr virus may replicate productively in mucosal epithelial cells but assume a latent state in B lymphocytes. Hence one cell type may serve as a repository which, following reactivation, seeds others. Even in a given cell type, permissiveness may be determined by the state of cellular differentiation or activation. For example, papillomaviruses complete a replication cycle only in fully differentiated epithelial cells. Again, HIV replicates in CD4+ T cells activated by an appropriate cytokine but remains latent in resting CD4+ T cells. Moreover, HIV enjoys a different type of association with cells of the monocyte/macrophage lineage, and other cell types in the body have been identified as further potential reservoirs of latent virus.

Non-Cytocidal Viruses

Arenaviruses and some retroviruses are examples of non-cytotoxic viruses that may establish persistent infections in

their reservoir hosts. In some natural pairings of virus and host, persistence is life-long with no chronic disease, while in others virus carriage declines slowly over time and transmission to further generations of reservoir hosts is required to complete the natural virus cycle. For example, Junin virus (the etiologic agent of Argentine hemorrhagic fever) produces a persistent infection, in its reservoir host, the vesper mouse, *Calomys musculinus*, with no disease but with long-term virus shedding in urine and saliva. Transmission from the mouse by aerosol and fomites to field workers, especially during harvest time, resulted for many years in outbreaks of severe hemorrhagic fever—the incidence has been greatly lowered in recent years by extensive use of vaccine.

Hepatitis B virus causes a non-cytotoxic infection of hepatocytes, and the extent of liver damage is dependent on the extent of immune-mediated cell damage (see below).

Evasion of the Immune Response

Cell-to-Cell Spread of Virus by Cell Fusion

Lentiviruses (e.g., HIV), paramyxoviruses (e.g., measles virus), and herpesviruses (e.g., cytomegalovirus) can cause neighboring cells to fuse together, enabling the viral genome to spread contiguously from cell to cell without ever being exposed to virus-specific circulating antibodies or cytotoxic T cells.

Sequestration of Virus in Anatomic or Physiological Sanctuaries

A number of persistent infections involve the central nervous system. The brain is insulated from most trafficking of lymphocytes by the blood–brain barrier; further, neurons express very little MHC antigen at their surfaces, thereby conferring some protection against lysis by cytotoxic T lymphocytes and the consequent release of viral antigens that might otherwise trigger an immune response. Herpesviruses, polyomaviruses, and lentiviruses are examples of viruses that can persist in the brain by taking advantage of its separation from normal immune surveillance. HIV is relatively protected not only in the brain but also in the epididymis.

The kidney is the other major site that frequently harbors persistent viruses, for example, cytomegalovirus and JC and BK polyomaviruses. These viruses are not acutely cytopathic, and perhaps because virus is released on the luminal surfaces of polarized epithelial cells immune surveillance is avoided.

An extreme case of inaccessibility to the immune system is employed by papillomaviruses causing warts; virus replication occurs in a stepwise fashion as skin cells mature—initial infection occurs in basal skin layers (*stratum germinativum*) but infectious virions are only

completed in the fully differentiated outer layers (*stratum granulosum* and *stratum corneum*) of the skin—there is little or no immune surveillance in such sites. Nevertheless, evidence of immune control of papillomavirus infections is demonstrated by the increased occurrence of warts in immunosuppressed individuals, and the tendency for multiple warts to all undergo spontaneous regression simultaneously.

Viral Antigenic Drift

In a number of persistent infections, notably HIV, mutations emerge at an extremely high rate because of the very high replication rate, for example, $\sim 10^{10}$ new virions per day, and the high error rate of the virion reverse transcriptase (approximately 1 base error per 10^4 nucleotides transcribed). This leads to continuing generation and then selection of antigenic escape mutants which are recognized only partly or not at all by the immune response specificity at the time. Such escape mutants often also have lessened replicative fitness, that is, they do not outcompete the original virus unless the latter's replication is being retarded by the immune response.

Virus-Induced Immunosuppression

Virus infections usually lead to an immune response ensuring recovery and elimination of the virus. However, a number of infections can be associated with suppression of the immune response. This suppression may either be generalized, that is, against many antigens, or it may only involve antigens specific to the virus in question. There are three major mechanisms involved:

1. Immunosuppression due to infection of immune effector cells,
2. Immunological tolerance induced by clonal deletion of T cells that would respond to the viral antigens,
3. Interference by viral products in various stages of the immune response.

Immunosuppression due to Infection of Effector Cells

Many viruses can replicate productively or abortively in cells of the reticuloendothelial system and it is noteworthy that these cells are often implicated in persistent infections. Lymphocytes and monocytes/macrophages and dendritic cells represent tempting targets for any virus, in that they move readily throughout the body, can seed virus to any organ, and are key players in the immune response.

A dramatic example of destruction of the body's immune system is provided by HIV, which replicates in CD4+ T lymphocytes, dendritic cells, and cells of the monocyte/macrophage lineage. In the latter, cytopathic

effects are minimal but the cell functions of phagocytosis, antigen processing/presentation, and cytokine production may be inhibited. DNA copies of the viral genome are integrated into the cellular DNA of CD4+ cells, and viral replication occurs only following activation of these lymphocytes by certain cytokines. CD4+ T cell death can occur by apoptosis, or following fusion with other T cells to form short-lived syncytia, or by lysis by CD8+ T cells. Clinically, immunosuppression is followed in a practical way by monitoring the number of CD4+ T cells in the blood, normally ranging from 500 to 1500/mm³. Virtual elimination of CD4+ T lymphocytes from the body eventually results in such profound depression of the immune response that the untreated patient dies from intercurrent infections caused by opportunistic pathogens, or from cancer (see Chapter 9: Mechanisms of Viral Oncogenesis and Chapter 23: Retroviruses).

Numerous other viruses temporarily induce generalized immunosuppression by abrogating the function of one or another arm of the immune response. Many viruses are capable of productive or abortive replication in macrophages. In many chronic viral infections virus replicates in reticuloendothelial tissues; this may impair several other key immunological functions, notably phagocytosis, antigen processing, and antigen presentation to T cells.

EB virus induces polyclonal activation of B cells which diverts the immune system to irrelevant activity.

Several viruses have been shown to grow productively or abortively in activated T lymphocytes. For example, measles virus replicates non-productively in T lymphocytes and suppresses Th1 cells (DTH). It has been known since the work of von Pirquet in 1908 that measles infection depresses skin responses to tuberculin and can reactivate latent tuberculosis. Other immunological abnormalities found during the acute phase of measles include spontaneous proliferation of peripheral blood lymphocytes and increased plasma interferon- γ . The depressed delayed-type hypersensitivity response, decreased NK cell activity, increased plasma IgE level, and increased soluble IL-2 receptor can persist for up to four weeks after the onset of the rash. These and other immunological abnormalities probably account for the susceptibility to secondary infections that cause most of the mortality during outbreaks of measles.

Induction of Immunological Tolerance

The probability of an acute infection progressing to chronicity is strongly age-related. In particular, congenital virus transmission to the fetus or neonate, whether transplacental or perinatal, greatly enhances the likelihood of persistent infection—this is the case with hepatitis B virus, rubella virus, cytomegalovirus, parvovirus B19, and others. This non-responsiveness may reflect “split

tolerance”—a B cell response may occur, but accompanied by a degree of T cell unresponsiveness. In the well-studied experimental model, lymphocytic choriomeningitis (LCM) virus infection, mice infected *in utero* do not mount a T cell response to the virus and the humoral immune response that is mounted does little to affect the course of the infection. This persistent infection can be reversed by adoptive transfer of sensitized CD8+ T cells, demonstrating their key role in recovery from this infection.

Interference with the Immune Response by Viral Products

Antibody-Induced Removal of Viral Antigens from Plasma Membranes of Infected Cells

Antibodies, being divalent, can bridge viral molecules on cellular membranes, bringing about “capping” followed by endocytosis of the antigen, thereby leading to a diminished display of viral antigens on the cell surface and diminishing their function as immunological targets. This phenomenon is readily demonstrable *in vitro* in “carrier cultures” of cells persistently infected with budding viruses; this phenomenon has been postulated to play a role in SSPE, where antibodies also down-regulate transcription of the measles viral genome.

Interference with MHC Antigen Expression or Peptide Display

Since CD8+ T cells recognize viral peptides bound to MHC class I antigens (and CD4+ T cells recognize them in the context of MHC class II antigens), viral persistence is presumably favored by reduction of peptide display on cell surfaces. The E1A protein of adenoviruses, the Tat protein of HIV-1, and E5 and E7 proteins of bovine papillomavirus all inhibit transcription of different genes coding for MHC components. The E7 protein of human papillomavirus 18 down-regulates the transcription of the TAP1 gene responsible for cytosolic transport of degraded peptides. Other viral proteins interfere with peptide generation (EBV, HHV-8, HIV-1) or transport (HSV, CMV, EBV), or the cell surface expression of MHC (adenoviruses, CMV, HTLV-I).

Down-Regulation of Cell Adhesion Molecules

Burkitt’s lymphoma cells carrying the EB virus genome display reduced amounts of the adhesion molecules ICAM-1 and LFA-3, and therefore bind T cells with lower affinity.

Viral Evasion of Host Cytokine Actions

Interferons are induced by infection with viruses and display a wide range of antiviral as well as immunomodulatory activities, as described in Chapter 5: Innate Immunity and Chapter 12: Antiviral Chemotherapy. However, many viruses code for proteins that block or subvert one or more

of the sequential steps in the interferon response (see Chapter 5: Innate Immunity). These actions are thought to facilitate persistence, for example, in the case of HCV and HIV-1, but also suppress the immune responses in some transient virus infections (for example SARS, West Nile virus, dengue, Ebola virus). Other viruses have developed the ability to counter other antiviral cytokines (e.g., an adenovirus gene-product partially protects infected cells against the action of tumor necrosis factor).

Blocking of the Immune Response by Non-Neutralizing Antibodies

Very high titers of antibodies are characteristic of many chronic viral infections, so much so that virus–antibody and antigen–antibody complexes accumulate at the basement membranes of renal glomeruli and other sites, causing a variety of immune-complex diseases. Yet the infection may not be eliminated and the complexes can remain infectious. A high proportion of these antibodies may be directed against viral proteins or epitopes that are not relevant to neutralization; by binding to virions these antibodies may block the attachment of neutralizing antibody by steric hindrance.

Production of Excess Viral Antigens

The chronic carrier state in hepatitis B is marked by the production of a huge excess of non-infectious particles of HBsAg thus mopping up neutralizing antibodies, a mechanism thought to overwhelm the body’s capacity for antibody production. It is possible some other chronic infections are also sustained by this strategy.

MECHANISMS OF DISEASE PRODUCTION

The continuing survival of a virus depends on an unbroken chain of transmission to, and replication in, new non-immune hosts; whether this produces pathology in the host, and/or clinical signs and symptoms, is often of little relevance to the survival of the virus in nature. Nevertheless the clinical consequence of the interactions between virus and host, namely clinical disease, is of course at the heart of medical virology.

Damage to Tissues and Organs Caused by Virus Replication

Viruses can damage cells in a number of different ways, including by mechanisms ending in necrosis, in apoptosis, or by expression of viral antigens that serve as targets for damaging immune attack (Chapter 5: Innate Immunity). The pathological effects at the level of tissues and organs are considered. The severity of disease in humans is not necessarily correlated with the degree of cytopathology produced by the virus *in vitro*. Many viruses that are cytotoxic in cultured cells do not produce clinical disease; for example,

some enteroviruses that cause a striking cytopathic effect in cultured human cells cause only inapparent infections in humans. Conversely, some viruses are non-cytocidal *in vivo* and in some instances in cultured cells but cause lethal disease—rabies virus (wild-type “street virus” strains) is a dramatic example. In some instances cell and tissue damage can occur without producing obvious disease; for example, a substantial number of liver cells can be destroyed without causing significant clinical signs. Damage to a proportion of cells in some organs and tissues may be of minor importance, e.g., in striated muscle, connective tissue, and skin, whereas it may be of major consequence if it occurs in key organs such as the heart or brain. Likewise, moderate levels of tissue edema may be unimportant in most sites in the body, but may have serious consequences in the brain if it leads to an increase in intracranial pressure, or in the lung where it may interfere with gaseous exchange, or in the heart where it may disrupt nerve impulse conduction.

Damage to the Epithelium of the Respiratory Tract

Respiratory viruses initially invade and destroy just a few epithelial cells, but initiate a “daisy-chain” process that progressively damages more and more epithelial cells and eliminates the protective layer of mucus. As viral replication progresses, large numbers of progeny virions are budded into the lumen of the airways. Early in infection, the beating of cilia, the primary function of which is to cleanse the respiratory tract of inhaled particles, may actually help to move released progeny virus along the airways, thereby fostering the spread of infection. As secretions become more profuse and viscous, the ciliary beating becomes less effective and ceases as epithelial cells are destroyed.

Studies of influenza and parainfluenza virus infections in experimental animals have shown virus spreading from the site of infection via contiguous expansion, not stopping until virtually all columnar epithelial cells along the major airways are infected. The result is a complete denudation of large areas of the epithelial surface (Fig. 7.10) together with the accumulation of transudates and exudates containing inflammatory cells and necrotic epithelial cell debris. A fatal outcome is invariably associated with one or more of three complications: bacterial superinfection (nurtured by the accumulation of fluid and necrotic debris in the airways), infection and destruction of the lung parenchyma and the alveolar epithelium, and/or blockage of airways that are so small in diameter that mucous plugs cannot be bypassed by forced air movements. Blockage of the airways is of most significance in the narrow-bore airways of the newborn. In all complications there is hypoxia and a pathophysiological cascade causing acidosis and an uncontrollable fluid exudation into the airways (acute respiratory distress syndrome—ARDS).

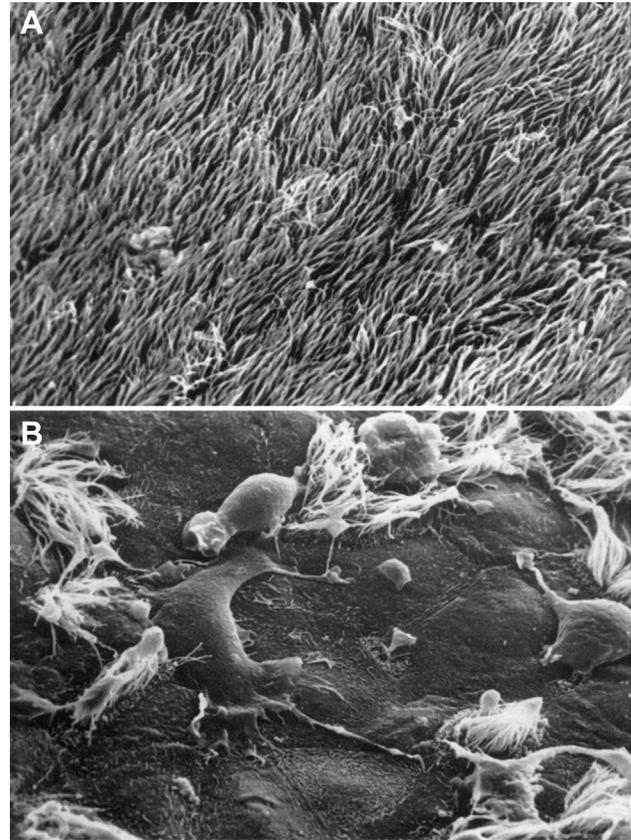


FIGURE 7.10 Organ cultures of tracheal epithelium, viewed from the luminal side by scanning electron microscopy. This has proved an excellent system to study the pathogenesis of respiratory virus infections. (A) Normal control culture; the epithelium appears as a thick carpet of fine projections—the carpet of cilia. (B) Six days after rhinovirus infection only a few tufts of cilia remain. The exposed non-ciliated sub-epithelial surface is quite smooth, consisting of flattened basal cells that will differentiate to reform the normal luminal surface. From Reed, S.E., Boyde, A., 1972 *J. Infection and Immunity* 6(1):68–76, with permission.

Degeneration of respiratory tract epithelial surfaces during influenza infection is extremely rapid, but so is regeneration. In studies of influenza in ferrets, for example, it has been shown that the development of a completely new columnar epithelial surface via hyperplasia and maturation of remaining transitional cells may occur within a few days. The transitional epithelium and the newly differentiated columnar epithelium that arises from it are resistant to infection, probably by virtue of interferon production and a lack of virus receptors. The role of other host defenses, including soluble factors such as mannose-binding lectins and lung surfactants, as well as macrophages, NK cells, IgA and IgG antibody, and T cell-mediated immune mechanisms in terminating the infection are discussed in Chapter 5: Innate Immunity and Chapter 6: Adaptive Immune Responses to Infection.

Damage to the Epithelium of the Intestinal Tract

The principal agents causing viral diarrhea are rotaviruses, caliciviruses, astroviruses, and certain adenoviruses (noroviruses). Infection occurs by ingestion, and the incubation period is usually very short.

Rotaviruses infect mature enterocytes at the tips of villi and cause a marked shortening and occasional fusion of adjacent villi. Infection generally begins in the proximal part of the small intestine and spreads progressively to the jejunum and ileum and sometimes to the colon. The extent of such spread depends on the initial virus dose, the virulence of the virus, and the host's immunological response. Diarrhea can occur in the absence of visible tissue damage, or, conversely, histological lesions can be asymptomatic. Factors contributing to fluid accumulation in the lumen of the gut and diarrhea include: (1) the reduced absorptive surface of the intestine as a result of the enterocyte damage, (2) the induction of chloride secretion by the rotavirus toxic protein NSP4, and (3) the impairment of intestinal disaccharidases leading to malabsorption of carbohydrates and osmotic shock. Acidosis develops due to the loss of Na⁺, K⁺, glucose, and water uptake, and also to the increased microbial activity associated with the fermentation of undigested milk. Acidosis can create a K⁺ ion exchange across the plasma membrane of epithelial cells, affecting cellular functions essential for the maintenance of normal K⁺ concentration. Hypoglycemia due to decreased intestinal absorption, inhibited glyconeogenesis, and increased glycolysis follow, completing a complex of pathophysiological changes that, if not promptly corrected by restoration of fluids and electrolytes, can result in death.

As infection progresses, the absorptive cells are replaced by immature cuboidal epithelial cells with greatly reduced capacity and enzymatic activity. However, these cells are relatively resistant to viral infection, so that the disease is often self-limiting if dehydration is not so severe as to be fatal. The rate of recovery is rapid, since crypt cells are not damaged and have a mitotic capacity.

Epithelial Damage Predisposing to Secondary Bacterial Infection

As well as having direct adverse effects, viral infections often predispose epithelia to secondary bacterial infections, increasing the susceptibility of the respiratory tract, for example, to bacteria that are normal commensals in the nose and throat (Fig. 7.10). Thus infections with influenza virus may destroy ciliated epithelia and cause exudation, allowing pneumococci and other bacteria to invade the lungs and cause secondary bacterial pneumonia, a not uncommon cause of death in the elderly. Conversely, proteases secreted

by bacteria may activate influenza virus infectivity by proteolytic cleavage of its hemagglutinin spike protein. Rhinoviruses and respiratory syncytial virus damage the mucosa of the nasopharynx and sinuses, predisposing the host to bacterial superinfection that commonly leads to purulent rhinitis, pharyngitis, sinusitis, and sometimes otitis media. Similarly, in the intestinal tract, rotavirus infection may lead to an increase in susceptibility to enteropathogenic *Escherichia coli* and other bacterial pathogens; the synergistic effect can lead to more severe diarrhea.

Physiological Changes without Cell Death

In some situations infected cells may show no obvious damage, but, as discussed in Chapter 5: Innate Immunity, specialized cells may function less effectively after infection. For example, lymphocytic choriomeningitis virus infection may appear harmless in mice, but less antibody may be produced by infected than by uninfected B cells. The same virus has no cytopathic effect on cells of the anterior pituitary gland; however the output of growth hormone is reduced and as a result infected mice are runted; likewise, persistent infection of insulin-producing islet cells in the pancreas may result in a lifelong elevation of blood glucose levels. Other viruses may indirectly alter the expression of cell surface MHC molecules, leading to destruction of the infected cells by immunological mechanisms; thus enhanced class II MHC expression after infection of glial cells by mouse hepatitis virus, perhaps due to the production of interferon- γ , may render these cells susceptible to immune cytotoxicity by cytotoxic T cells.

Immunopathology—Cell Damage Caused by Immunological Processes

The immune response is an essential part of the pathogenesis of most virus diseases. Infiltration of lymphocytes and macrophages, with accompanying release of cytokines and inflammatory mediators, is a regular feature of viral infection that assists in recovery from infection. However these processes can also be a major cause of cell and tissue damage in some infections, and many of the common symptoms of viral disease (fever, erythema, edema, and enlargement of lymph nodes) have an immunological basis. When pathological changes are ameliorated by immunosuppression, it can be assumed that underlying immunopathology is making an important contribution to the disease process.

In particular, for non-cytolytic viruses it is likely that immune mechanisms are the main cause of disease. In most cases these mechanisms involve activated T cells, although there are some examples due to antibody or an exuberant innate immune response.

Immunopathology Resulting from the Activation of Cytotoxic T Lymphocytes

This mechanism involves both lymphocyte and macrophage accumulation at sites of virus replication, together with local secretion of proinflammatory cytokines by these cells. Of course, cell-mediated immune responses are also an important contributor to recovery from viral infections (see Chapter 6: Adaptive Immune Responses to Infection), as becomes evident if they are abrogated by cytotoxic drugs, or are absent as is the case with some immunodeficiency diseases.

The classic model of death due to a cell-mediated immune response is lymphocytic choriomeningitis in adult mice inoculated intracerebrally. The virus replicates harmlessly in the meninges, ependyma, and choroid plexus epithelium for about a week until a CTL-mediated immune response develops, causing severe meningitis, choroiditis and ependymitis, and cerebral edema, convulsions and death. Experiments with knockout mice, and with chemical immunosuppression and adoptive transfer of T cells, have clearly confirmed that CTLs are necessary for the development of disease. Normally CTLs help to control infection, but within the rigid confines of the skull this response is fatal.

A lethal pneumonia develops when some inbred strains of mice are infected with certain strains of influenza virus by the intranasal route. Adoptive transfer of influenza virus-primed CD8⁺ cytotoxic T cells protect mice against intranasal challenge, but CD4⁺ Th1 cells actually accelerate their demise. Coxsackie B virus infection of mice is manifested as myocarditis, but in transgenic mice lacking the perforin gene, the disease takes a mild course, indicating that both intrinsic virus-induced damage and cytotoxic lymphocytes contribute to the pathology.

Hepatitis B is a clear example of liver damage as a result of specific immune responses to viral proteins. Transgenic mice expressing hepatitis B envelope proteins show little liver damage unless injected with hepatitis B-specific CTLs; this causes hepatocyte damage and inflammatory cell infiltration, characteristic of acute viral hepatitis. In humans, the degree of liver damage is governed by the strength of the immune response. Patients with marked immunosuppression can express high levels of virus in the absence of significant liver damage: patients with a strong immune response clear the virus, but those with an intermediate response show continuing liver damage and viral persistence. Any restoration of immune competence can induce pathology; for example, in HBV/HIV co-infected patients there is partial recovery of immune competence with a risk of “flares” of liver damage, after patients are treated with anti-retroviral drugs.

The maculopapular rash of measles involves virus infection of endothelial cells in the superficial layers of the dermis, spread of infection to overlying epithelial cells, vascular dilatation, and infiltration of CD4⁺ and CD8⁺ T

cells and macrophages into sites of replication. Individuals with deficiencies in cellular immunity may develop measles without a rash, but the disease in such patients is especially severe.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

In experimental cell culture systems, when antibody combined with viral antigen is present on the surface of infected cells, there can be a sensitization in which Fc-receptor-carrying K (killer) cells, together with polymorphonuclear leukocytes and/or macrophages, cause damage. Alternatively, the binding of antibodies can activate the complement system, leading to cell lysis. While it has been clearly demonstrated that virus-infected cells *in vitro* and in some experimental animal models are readily lysed by ADCC, its importance in human viral diseases remains unclear.

Immune Complex-Mediated Disease

Antigen–antibody reactions cause inflammation and cell damage by a variety of mechanisms. If the reaction occurs in extravascular spaces the result is edema, inflammation, and infiltration of polymorphonuclear leukocytes, which may later be replaced by mononuclear cells. This is a common cause of mild inflammatory reactions. Such immune complex-mediated reactions constitute the classical Arthus response and are of major importance, especially in persistent viral infections. If these occur in the blood, the result is circulating immune complexes, which are found in most viral infections. The fate of the immune complexes depends on the ratio of antibody to antigen. When there is a large excess of antibodies, each antigen molecule is covered with antibody and removed by macrophages bearing receptors for the Fc component of the antibody molecules. Alternatively, if the amount of antigen and antibody is about equal, lattice structures develop into large aggregates that are removed rapidly by the reticuloendothelial system. However, in antigen excess, as occurs in some persistent infections, viral antigens and virions are continuously released into the blood but the antibody response is weak and the antibodies are of low avidity or are non-neutralizing. Complexes may continue to be deposited in small blood vessels and kidney glomeruli over periods of weeks, months, or even years, leading to an impairment of glomerular filtration and eventually to chronic glomerulonephritis.

A classic example is lymphocytic choriomeningitis infection of mice infected *in utero* or as neonates. Viral antigens are present in the blood, and small amounts of non-neutralizing antibodies are formed, giving rise to

immune complexes which are progressively deposited on renal glomerular membranes; the end result may be glomerulonephritis, uremia, and death. Circulating immune complexes may also be deposited in the walls of the small blood vessels in skin, joints, and the choroid plexus, where attracting macrophages and activate complement. Prodromal rashes, which are commonly seen in exanthematous diseases, are probably caused in this way. A more severe manifestation of the deposition of antigen–antibody–complement complexes in capillaries is erythema nodosum (tender red nodules in the skin); when small arteries are involved, as is occasionally seen in patients with hepatitis B, the result is periarteritis nodosa.

In addition to these local effects, the mobilization of soluble mediators induced by antigen–antibody complexes may generate late systemic reactions, such as fever, malaise, anorexia, and lassitude common to most viral infections. Fever is attributed to interleukin-1 and tumor necrosis factor produced by macrophages, and possibly to interferons.

Rarely, systemic immune complex reactions may activate the enzymes of the coagulation cascade, leading to histamine release and an increase in vascular permeability. Fibrin may be deposited in the kidneys, lungs, adrenal glands, and pituitary gland, causing multiple thromboses with infarcts and scattered hemorrhages—a condition known as disseminated intravascular coagulopathy (DIC).

Systemic Inflammatory Response Syndrome (“Cytokine Storm”)

The immune response to an infection is normally modulated to a level proportionate to the extent of the infection, and is down-regulated once the infection has resolved. However, in some infections there is a dramatic and large-scale release of inflammatory cytokines accompanied by stress mediators that leads to overwhelming, even lethal, damage to the host. Also known as a “*cytokine storm*,” this is particularly seen with newly emerging or zoonotic infections, for example, SARS, Ebola hemorrhagic fever, hantavirus pulmonary syndrome, avian H5N1 influenza. It possibly was one key to the exceptional virulence of the 1918 influenza virus strain, the strain that caused the great global pandemic. The pathology associated with a “cytokine storm” includes microvascular damage, pulmonary edema, capillary leakage, hypotension, and organ failure.

Autoimmunity

Antibodies reactive against the host’s own proteins (i.e., autoantibodies) can be detected frequently in viral infections, although usually only transiently and in low titer. In one study, 4% of a large panel of monoclonal antibodies raised against several viruses were found to react with normal tissues. For example, a monoclonal antibody

directed against the neutralizing domain of Coxsackievirus B4 also reacted against heart muscle; this virus is known to target muscle, including cardiac muscle, and to cause myocarditis.

One likely explanation for this widespread cross-reactivity is that viral proteins share identical or near-identical sequences of 6 to 10 amino acids with cellular proteins far more frequently than would be predicted by chance. For instance, there is partial homology of amino acid sequences (*molecular mimicry*) between myelin basic protein (MBP) and several viral proteins. Alternatively, viral infections may lead to exposure to the immune system of cellular antigens normally sequestered or in some other way hidden. It has also been proposed that some T cells may carry dual T cell receptors, and as a consequence an autoreactive T cell normally depleted in the thymus may be activated should the host be exposed to a viral antigen also recognized by a second T cell receptor on the same cell.

Such mechanisms may be involved in the neurological disorders associated with animal lentivirus infections, visna, and caprine arthritis-encephalitis, and in the rare occurrence of post-vaccinal encephalitis in humans. Inoculation of a neuritogenic protein of peripheral nerve myelin can cause the experimental equivalent of Guillain-Barré syndrome. There is mimicry between the epitope involved (P2) and a sequence in the influenza virus NS2 protein. Ordinarily, this non-structural protein is removed from influenza virus vaccine during purification; failure to do this in some batches of swine influenza vaccine used in the crisis program mounted in the United States in 1976 may have accounted for the apparent increase in the incidence of Guillain-Barré syndrome at that time.

Post-infectious encephalomyelitis is a rare condition (about one in every thousand cases) that follows a few weeks after acute measles, and even more rarely following varicella, mumps or rubella infection or vaccinia vaccination. The pathology is predominantly demyelination without neuronal degeneration—unlike lesions produced by the direct action of viruses on the central nervous system. Allied with the failure to recover virus from the brain of fatal cases, this has led to the view that post-infectious encephalitis is probably an autoimmune disease. Myelin basic protein (MBP) can be found in the cerebrospinal fluid, and, at least in the case of measles, anti-MBP antibodies and T cells can be detected in the blood. Theoretically, autoimmune damage can continue long after the virus that triggered the response has been cleared from the body. Once the cross-reactive viral amino acid sequence has induced a humoral and/or cellular immune response that brings about damage to normal tissue, proteins that are normally sequestered may be exposed to the immune system. If the cellular protein is capable of immune recognition by the host, there may be a chain reaction of progressive damage. Implicit in this hypothesis is the notion of molecular mimicry being responsible for autoimmune

TABLE 7.8 Potential Mechanisms of Induction of Autoimmune Disease by Viruses

Molecular mimicry: viral protein elicits humoral and/or cellular immune response that cross-reacts with identical or similar epitope fortuitously present on a cellular protein

Activation of bystander autoreactive cells. Ongoing local inflammation may result in liberation of self-antigens that are not normally exposed to the immune system: these then become targets for bystander autoreactive T cells. This may also lead to “epitope spreading” to involve wider areas. Since such T cells are thought to have low affinity for self-antigen, viral infection itself may also contribute an adjuvant effect

Immortalization of polyclonal autoreactive effector cells or antigen-presenting cells, by a virus, e.g., EB virus

T cells expressing dual T cell receptors; an autoreactive T cell that would normally be thymically depleted might be activated if it contacts a viral antigen recognized by a second TCR that it is carrying

Induction of MHC antigens by cytokines: virus induces production of interferon γ and tumor necrosis factor, which induce expression of MHC class II protein on brain cells that do not usually express it, for example, glial cells, enabling them to present antigens such as myelin to T cells

Viral destruction or down-regulation of T cells that normally suppress immune response to self-proteins

disease while normal immunological tolerance is somehow deregulated.

Humans suffer from numerous autoimmune diseases, ranging from multiple sclerosis to rheumatoid arthritis. Most are chronic and difficult to treat, many are common, and most are of unknown etiology. Viruses are major suspects as triggers for these diseases, but definitive proof has yet to be produced, and this problem continues to be an important area for research. Current theories for which there is some evidence are summarized in [Table 7.8](#).

VIRUSES AND IMMUNOSUPPRESSION

Immunosuppression Caused by Viral Infection

Suppression of one or more components of the immune system is seen during the course of many viral infections. In acute infections this effect is almost always transient, but it may or may not play a part in the pathogenesis of the infection in question. In a number of persistent infections, virus-induced immunosuppression may be important in the establishment and maintenance of virus persistence. This is discussed in a previous section.

Viral Infections in Immunocompromised Patients

Impairment of the immune system can exacerbate disease or predispose to superinfection with other infectious agents. An immunocompromised state may be due to genetic defects, such as in congenital agammaglobulinemia. Most commonly, however, immune dysfunction is due to some other disease or circumstance, especially lymphomas, leukemias, infection with HIV, chemotherapy, or radiotherapy employed to treat tumors or to prevent organ transplant rejection.

Patterns of viral infections in persons with immune dysfunction depend on the particular virus and which arm of the immune system is defective. Many viral infections in immunocompromised subjects follow the disease pattern seen in normal persons, differing only in severity. Occasionally, if the disease is largely immunopathological, the disease may actually be milder—for example, in immunosuppressed renal transplant patients infected with hepatitis B there may be less liver damage. Usually, however, the immunocompromised patient suffers more severe and/or prolonged disease, and sometimes relatively innocuous viruses can prove lethal. For example, measles in patients with impaired cell-mediated immunity may cause giant cell pneumonia, sometimes several months after the acute infection, and often with fatal consequences. Immunosuppressed patients are also more likely to suffer generalized infections with viruses that usually cause localized disease; for example, herpes simplex and varicella-zoster. Immunocompromised individuals are not only subject to exogenous infections but suffer increased reactivation of latent viruses, especially herpesviruses, but also adenoviruses and polyomaviruses. These situations are described in more detail in the chapters dealing with specific viral diseases in Part II of this book.

Finally, immunosuppression commonly leads to reactivation of persistent infections. We should distinguish the reactivation of virus replication in a latently infected cell, at the cellular level, and the recrudescence of clinical disease. Examples of clinical reactivation are listed in [Table 7.9](#).

VIRAL VIRULENCE AND HOST RESISTANCE TO INFECTION

It is a common observation that different individuals in a family or community may show different levels of severity of disease when infected at the same time from a common source. Similarly, different strains of the same common virus may cause disease of different levels of severity (e.g., the H1N1 pandemic influenza strain that swept the world in 2009 was highly transmissible but of low virulence, while the H5N1 avian virus seen mostly in Asia in recent years is very poorly transmissible between humans but causes significant mortality). Influenza virus studies clearly distinguish the viral quality of *transmissibility* from that

TABLE 7.9 Examples of Clinical Reactivation of Persistent Viral Infections in Humans

Circumstances	Virus	Features
Old age	Varicella virus	Rash of shingles
Pregnancy	Polyomavirus JC, BK	Viruria
	Cytomegalovirus	Replication in cervix
	Herpes simplex virus 2	Replication in cervix
Immunosuppression in organ transplantation, autoimmune disease, malignancy, HIV	Herpes simplex virus 1, 2	Vesicular rash, potential for systemic spread
	Varicella virus	Vesicular rash (chickenpox or shingles), potential for systemic spread
	Cytomegalovirus	Fever, hepatitis, pneumonitis, GI tract disease
	Epstein-Barr virus	Increased shedding from throat, PTLD ^a
	JC polyomavirus	Viruria very common, PML ^b
	BK polyomavirus	Interstitial nephritis, hemorrhagic cystitis
	Hepatitis B virus	Viremia, hepatitis flare
	Adenoviruses	Increased shedding/disease in different organs
HIV/AIDS	Human papillomaviruses	More extensive warts (skin/anogenital)
		HPV-related cancers
	Cytomegalovirus	Retinitis, colitis, pneumonitis
	JC polyomavirus	PML ^b
	Epstein-Barr virus	Increased shedding from throat, non-Hodgkin's lymphoma
	HHV-8	Kaposi's sarcoma
	Herpes simplex virus 1, 2	Spreading vesicular rash, may be chronic
Varicella-zoster virus	Shingles	

^aPTLD, post-transplantation lymphoproliferative disease.

^bPML, progressive multifocal leukoencephalopathy.

of *virulence*—with other viruses this distinction is usually harder to define. Such studies also highlight the fact that the severity of an infection is influenced by both virus- and host-determined factors.

Genetic Factors Affecting Viral Virulence

Viral virulence is influenced by viral genes in four categories: (1) those that affect the ability of the virus to replicate, (2) those that affect host defense mechanisms, (3) those that affect tropism, spread throughout the body and transmissibility, and (4) those that encode or produce products that are directly toxic to the host.

It has been a longstanding goal of virologists to understand and to predict the mechanisms underpinning the

virulence of pathogenic viruses. In addition to the clinical aim of a better understanding of viral pathogenesis, such knowledge allows us a rational basis for designing avirulent virus strains as attenuated live-virus vaccines. However, reductionist attempts to identify a “virulence gene” (or genes) have usually been inconclusive, as virulence has usually turned out to be based on a co-operative effect between a number of different genes (often called “*the constellation of genes*” encoding virulence and transmissibility factors). For example, after the highly virulent 1918 pandemic influenza virus (an H1N1 virus) was reassembled from permafrost-frozen cadavers and from formalin-fixed tissue samples taken during the epidemic, genetic reassortants were constructed containing genes from the epidemic virus and an avirulent H1N1 strain (TX/91); the virulence of TX/91

increased as more of its genes were replaced by genes from the pandemic strain. No single gene was found to be wholly responsible for the virulence of the epidemic virus, but the hemagglutinin gene and genes of the polymerase complex contributed most to the virulence phenotype.

Genes affecting the ability of the virus to replicate. One well-studied example comes from the comparison of wild-type and vaccine strains of poliovirus. It was clearly shown that virulent and attenuated viruses differed in only a small number of nucleotides. Infectious genomic cDNA constructs containing different combinations of wild-type and vaccine strain sequences were tested for neurovirulence in monkeys and in transgenic mice expressing the poliovirus CD155 receptor. Attenuation was shown to be determined primarily by mutations within the internal ribosome entry site (IRES) within the 5'-UTR (see Chapter 32: Picornaviruses), but there was also a contribution from mutations within the capsid region of the viral genome. The mutations in the IRES altered stem-loop structures and reduced the efficiency of translation of poliovirus RNA, while the capsid mutations may have led to impaired binding to the cellular receptor and reduced stability of the capsid.

Genes affecting virus spread or tropism. Influenza virus replication requires cleavage of the hemagglutinin precursor protein HA0 into its two subunits HA1 and HA2. Studies of avian influenza strains have shown that avirulence is associated with the presence of a monobasic cleavage site that is susceptible to cleavage by trypsin-like proteases present only in the respiratory tract (mammals) and gastrointestinal tract (birds). In contrast, highly pathogenic avian influenza virus strains contain a polybasic cleavage site that is susceptible to cleavage by furin, an enzyme widespread in the body, thereby promoting systemic infection.

Influenza virus tropism is also dependent on the distribution of appropriate sialic acid virus receptors; human viruses preferentially bind to α 2,6-linked sialic acids, while avian viruses preferentially bind to α 2,3-linked sialic acids. In humans, α 2,6-linked sialic acid receptors are predominant on ciliated epithelial cells and goblet cells of the upper respiratory tract, whereas α 2,3-linked sialic acid receptors are predominant on non-ciliated bronchiolar cells and alveolar type II cells in the lower respiratory tract. Clinical observations indicate that human seasonal influenza viruses predominantly infect the upper respiratory airways and are readily transmissible, while avian viruses acquired directly from birds (e.g., H5N1) often progress to severe lower respiratory tract disease in humans, but are not as transmissible between humans; these observations are consistent with the above patterns of receptor usage operating as virulence mechanisms.

Genes that affect host defense mechanisms. A number of viruses, particularly the large DNA viruses, encode proteins that interact with host defense systems. These include proteins that mimic cytokines or growth factors, and proteins that mimic virus receptors. Separately, several

viruses down-regulate the expression of MHC molecules at the surface of infected cells, thereby interfering with the elimination of infected cells by CD8⁺ T cells. Hepatitis C virus is a poor inducer of interferon α , and work with the recently developed cell culture system for HCV has shown two mechanisms for this—(1) cleavage by the HCV NS3/4A protease of the cell mitochondria adapter MAVS involved in interferon induction, and (2) HCV-mediated suppression of protein translation by induction of phosphorylation of the eIF2 α translation initiation factor.

Genes coding for proteins that are directly toxic. In contrast to bacterial infections, examples of this mechanism have rarely been found in viral infections, perhaps reflecting the fact that a successful virus needs to maintain the viability of its host cell long enough to allow its replication. The best documented example is the non-structural glycoprotein NSP4 of rotaviruses. This protein inhibits a Na⁺-glucose luminal co-transporter which is required for water resorption in intestinal cells, and also increases intracellular calcium levels by inducing a phospholipase C-dependent calcium signaling pathway. Both effects enhance fluid loss.

Host Genetic Factors Affecting Virulence

It has long been known that different animal species show different degrees of susceptibility to the same virus. The benign course of B virus (Cercopithecine herpesvirus 1, formerly called herpesvirus simiae) infection in macaques compared with the virulence of the same virus for humans is a clear example. In general, there is a tendency for low virulence to be seen with a virus that has been long established in a particular host, compared with high virulence when the same virus is introduced into a new host species; this is a frequent observation in cross-species transfer of viruses and with emerging zoonotic infections of humans.

In some examples using inbred strains of susceptible and resistant mice, susceptibility genes have been mapped and functions identified. Not surprisingly, associated susceptibility has been shown as linked to genes for MHC haplotypes or components of the interferon system, reflecting the critical role played by the *balance* between virus invasion and host defense in determining the severity of an infection. Mouse strains that are resistant to flavivirus infection were found to have a mutation in the *flv* gene, which was subsequently shown to encode 2'-5'-oligo(A) synthetase, an interferon-induced enzyme that activates RNase L resulting in the breakdown of viral and host mRNA. One well-studied human example involves the chemokine receptor CCR5 which acts as a co-receptor for infecting strains of HIV; individuals carrying a mutation in this gene show greatly increased (but not complete) resistance to HIV infection.

In another example, humans who are predisposed to herpes encephalitis have been found to have mutations in either of the genes *TLR3* or *UNC-93B*, both of which

code for proteins that affect interferon production. *TLR3* appeared not to be involved in resistance to other infections but is vital for natural immunity to herpes simplex virus infection of the CNS, implying that neurotropic viruses may contribute to maintaining *TLR3* through evolutionary time.

However, in most human viral infections the reasons why different individuals respond differently to the same virus remains a mystery, very likely grounded in multifactorial subtle variations in innate and adaptive immune responsiveness.

Physiological and Other Host Factors Affecting Virulence

Host factors such as age, nutritional state, pregnancy, immunity from prior exposure, and co-infections all contribute to the outcome of virus infections. In natural infections of human populations it may be difficult to tease out the relative contribution of co-existing effects, but animal models have allowed the study of individual factors.

Age. For many years virologists used inoculation of suckling mice as a sensitive way to search for new viruses, particularly arboviruses and enteroviruses, many of which do not infect adult mice. In humans, more severe outcomes are often seen in the very young and the very old, and different outcomes in patients of different ages are well described. For example, hepatitis A infection is more likely to lead to the development of fulminant hepatitis in the elderly (Table 7.10).

An infection in early infancy with many viruses leads to more severe, sometimes devastating, disease because the maturing immune system is not yet able to respond as well as in older patients. Transplacentally acquired maternal antibody can provide a short-term protective umbrella, so such examples tend to be more severe if the mother is undergoing a primary infection and the infant is born without

maternal antibodies. The tendency for very young infants with respiratory syncytial virus infection to suffer from severe bronchiolitis has also been ascribed to the narrower airways in the very young. Other viruses cause more severe infections or more complications in adults, for reasons that are not well understood. In these examples, subclinical infection acquired inadvertently in childhood can in fact give immunological protection against later adult disease.

Hepatitis B virus provides a well-studied example. Persistent infection almost invariably follows infection in infancy, but in only 5 to 10% of infections acquired by adults; intermediate rates of persistence are seen at intermediate ages. This effect again is thought to be due to immaturity of the immune system. In the duck hepatitis B model, the transition from virus persistence to virus clearance occurs in young ducklings at quite a sharply demarcated age—by increasing the dose of virus inoculated, the age point favoring persistence versus clearance is shifted to older and older ducks. This highlights the principle that the outcome of virus exposure reflects a balance between the rates of virus replication and the immune response.

Nutritional state. Malnutrition can interfere with most or all of the mechanisms that serve as barriers to the progress of virus disease. It has been repeatedly demonstrated that severe nutritional deficiencies interfere with the generation of antibody and cell-mediated immune responses, with the activity of phagocytes, and with the integrity of skin and mucous membranes. However, often it is impossible to disentangle adverse nutritional effects from other adverse factors found in developing communities. Moreover, just as malnutrition can exacerbate viral infections, so viral infections can exacerbate malnutrition, especially if repeated severe diarrhea is a feature, and especially in the first year of life.

Children with protein deficiency of the kind found in many parts of Africa are highly susceptible to measles. All the epithelial manifestations of the disease are more severe, and secondary bacterial infections cause life-threatening disease of the lower respiratory tract as well as otitis media, conjunctivitis, and sinusitis. The skin rash may be associated with numerous hemorrhages, and there may be extensive intestinal involvement with severe diarrhea, which exacerbates the nutritional deficiency. The case–fatality rate is commonly 10% and may approach 50% during severe famines, and a debilitating chronic malnutrition/malabsorption syndrome can occur among survivors.

Other factors. In experiments with myxoma virus in rabbits fever has been shown to increase protection against disease; blocking the development of fever with salicylates was shown to increase mortality. Pregnancy can trigger the reactivation of some persistent viruses, particularly herpesviruses and polyomaviruses, thereby adding to the risk of the neonate acquiring herpes simplex infection during birth. Certain infections are markedly more severe in pregnancy (e.g., hepatitis E, smallpox) (Box 7.1).

TABLE 7.10 Effect of Age on Severity of Infection

- Many infections are more severe in infancy than in older children: rotavirus, respiratory syncytial virus, herpesviruses, Coxsackieviruses (e.g., due to less mature immune system, no prior immunity, anatomical factors in the respiratory tract)
- Some important infections are *more* severe in older children or adults: polio, hepatitis A, measles, Epstein-Barr virus, varicella, mumps (orchitis)
- Hepadnaviruses: > 90% of infections in infancy lead to persistent infection, only 5% to 10% of adult infections become persistent
- Some chronic infections progress more rapidly in people over the age of 30 to 40: HIV, hepatitis C
- Many acute virus infections cause greater morbidity and mortality in elderly persons, in part due to declining immunocompetence and co-morbidities (influenza)
- In some infections with a very late outcome, the patient may die first from other causes (hepatitis C, human T cell leukemia virus)

BOX 7.1 Important definitions

Pathogenicity is defined as the absolute ability of an infectious agent to cause disease/damage in a host—an infectious agent is either pathogenic or not.

Virulence is defined as the relative capacity of an infectious agent to cause disease/damage in a specific host. *Virulence* must be measured relatively, comparing one infectious agent to another, or comparing our historical experience with one disease or infectious agent to another (i.e., “virus A is more *virulent* than virus B in a specified host”). The terms *pathogenicity* and *virulence* do not refer to the properties of infectiousness, infective titer, or transmissibility, only to the capacity to cause disease or damage in a host.

Virulence is dependent upon many factors including the age, sex, species, and physical condition of the host involved, the route of infection, the strain of the virus of interest, etc. For example, many viruses are relatively avirulent in a host species in which they have been endemic for a long time, but cause severe disease when introduced into a new host species (host range extension—“host species jumping”). This is an important principle in understanding the emergence of zoonotic infections in humans (Chapter 15: Emerging Virus Diseases). Furthermore, there are well-studied examples where a virus may be virulent when introduced by one route, for example, intracerebral inoculation, but harmless when given by another route, for example, intraperitoneally.

The *virulence* of a virus can be quantitated, at least in experimental terms, by titrating serial dilutions in groups of (ideally inbred) animals, and recording the virus dilution that causes an endpoint of death or disease in 50% of the animals (the lethal dose for 50% of subjects, the LD₅₀; or the infectious dose for 50% of subjects, the ID₅₀). Other endpoints that can be used are the time to death or the time to the appearance of clinical signs, weight loss, or fever (to accord with modern standards for humane care of experimental animals). Assessment of the virulence of a virus in humans cannot be made in such a controlled experimental way, but often becomes evident when observing outbreaks of acute infection. Interpretation is more complicated in chronic infections—for example, the appearance of a new strain, or a higher viral load, of HIV in a patient whose clinical condition is worsening, may indicate that a new strain or higher virus replication is *causative* of the increasing disease, or it may be a *result* of increasing immunosuppression.

CO-INFECTIONS

Co-infections with more than one virus are increasingly recognized, and are of increasing practical concern in an era of greatly improved options for antiviral drug use. At one end of the spectrum, use of better diagnostics, particularly rapid PCR-based tests for respiratory and gastrointestinal viruses, has revealed that multiple infections are not at all unusual, nor unfortunately is the nosocomial acquisition within hospital of further virus infections by a child after being admitted to hospital for one initial infection. This can create ambiguity in ascribing a patient’s clinical disease to any one particular virus detected by the diagnostic laboratory, and necessitates

careful case-control studies of populations at risk with and without disease before a newly discovered virus can be definitively implicated as a new pathogen.

At the other end of the spectrum, many patients have been exposed to HIV, HCV, and/or HBV, and dual or even triple infections are increasingly reported. Management of these patients poses additional challenges. In patients with HBV or HCV infection, the additional presence of HIV infection produces clinical outcomes that might be expected from a diminished immune response: with HBV, higher levels of virus replication and milder inflammatory liver disease, and with HCV a lower rate of clearance of the HCV infection. However, in both situations liver disease frequently progresses more rapidly through fibrosis to cirrhosis and brings a worsening prognosis. Conversely, the additional presence of HBV or HCV does not appear to significantly influence the course of HIV infection. Selection of optimal antiviral drug regimens requires consideration of the patient’s history of prior treatment, resistance patterns of the patient’s virus, and the multiple effects of some drugs against more than one virus. Successful treatment of HIV infection with anti-retroviral drugs carries the risk of “immune restoration disease,” which may involve recruitment of both antigen-specific and non-antigen-specific mononuclear cells to the liver, possibly mediated by interferon- γ . With both HBV and HCV, this can lead to flares of inflammatory liver disease, which can progress to hepatic decompensation in patients with cirrhosis.

FURTHER READING

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